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STUDIES ON THE BIOLOGY OF STREPTOCOCCUS.

I. Antigenic Relationships between Strains of Streptococcus hæmolyticus.

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(From the Hospital of The Rockefeller Institute for Medical Research.)

(Received for publication, June 1, 1919.)

The importance of the problem of the systematic classification of bacteria for the proper understanding and control of infectious diseases is becoming increasingly evident. Such study is necessary not only in the elucidation of the biological relationship existing between varieties of the same species of bacterium, but is also essential to the working out of epidemiological problems and to the development of knowledge useful in the effort to control infectious diseases by means of specific therapeutic and prophylactic measures. Bacteria closely resembling those responsible for the pathological process in many acute infections have been found to be present and to live, apparently without harm to the host, on the mucous membranes of a large proportion of normal individuals. The resemblance of the pathogenic to the harmless variety of microorganism is frequently so close that in many instances tests of particular specificity are required to show the existing biological differences. In fact, the problem in etiology today is to determine not only the bacterial species causing a given disease, but in addition the number of varieties of the same species that are pathogenic, and whether common and important non-pathogenic varieties exist. The study is one of varying complexity, and methods suitable to one species do not give the desired information when applied to another.

The purpose of such studies may be broadly defined as an effort to relate fixed and determinable characteristics of bacteria to pathogenicity. Though fluctuating variations of bacteria probably occur, it seems not unlikely that in most diseases a sufficiently constant equilibrium has been attained to justify the usefulness of the effort. The

methods employed in these studies are numerous. In some species morphological and cultural characters give important though somewhat limited information, and in many the biochemical reactions are of great significance. The most serviceable method, however, for obtaining the particular kind of knowledge desired is the study of immunological relationships. For some as yet unexplained reason the latter specific reactions are very constant among the pathogenic varieties. In the following study of *Streptococcus hæmolyticus* chief dependence has been placed on the knowledge obtained from the study of these immunity reactions.

In the various classifications of the streptococcus group as a whole that have been proposed, the custom has been to consider the strains that effect hemolysis of red blood cells as constituting a single or unit type (1). The validity of this assumption has been questioned and there has been much study and discussion of the probability of the existence of variations within this group, some evidence of which has been obtained from the study of sugar fermentations (2). Recent studies (3) indicate that certain broad lines of differentiation may be shown between hemolytic streptococci of human origin, and those of bovine origin whether found in milk or cheese. Hemolytic streptococci of the human type are usually found in association with some pathological process such as puerperal sepsis, septicemia, erysipelas, bronchopneumonia, or other conditions, and hemolytic activity is generally considered as an indication of pathogenicity. With the development of our knowledge (4), however, these organisms have been found with increasing frequency when no pathological lesion has been apparent. Investigators for many years have been interested in these strains, and discussion has centered about the unity or multiplicity of the group (5). The evidence in general favors the belief that hemolytic streptococci pathogenic for human beings comprise a single type. In the present paper it will be shown that by the use of properly controlled immune reactions differential characters between individual strains can be shown to exist.

During the winter of 1917–18 in the United States, there occurred in numerous localities a great increase in the incidence of a previously rather infrequent type of bronchopneumonia. The highest morbidity rate and earliest appearance of this disease were in military cantonments. In the spring of 1918, however, the type of infection under consideration was commonly observed in the civilian population. The disease first appeared as a secondary pneumonia following measles, but soon instances of apparently primary infection of the lungs were observed. Numerous studies of the bacteriology

of this condition have demonstrated that the infectious agent responsible for the lung lesion was in almost every instance *Streptococcus hæmolyticus*. As a result of the widespread incidence of the disease the latter organism became extensively distributed and was frequently found as a secondary invader in acute lobar pneumonia, and as a common inhabitant of the normal throat.

The material used in the present study was obtained from the military establishments in the neighborhood of Fort Sam Houston, Texas. The sources of the individual strains were the throats of patients suffering from acute measles, the sputum of patients with bronchopneumonia both primary and secondary to measles, pathological material obtained from cases of bronchopneumonia and acute lobar pneumonia, and the throats of healthy individuals who had been directly or indirectly exposed to infection in a variety of ways.

All the strains of hemolytic streptococcus employed in this study possess the typical characteristics of the group. They are hardy organisms and grow readily in meat infusion broth and on blood agar slants. They survive for many months when grown for 18 hours in rabbit blood broth and subsequently placed at refrigerator temperature. In meat infusion broth the growth has been of two types —a granular sediment with clear supernatant fluid and a flocculent sediment with turbidity throughout the remainder of the tube. All the organisms are strongly Gram-positive, grow in chains of varying length, and are bile-insoluble. Capsule formation has not been observed. On plates two types of colonies are seen—a small, round, smooth colony and a moist ameboid colony with a slightly roughened surface. All the strains are actively hemolytic, showing a wide zone of hemolysis on the surface of rabbit blood agar plates; hemolysis is complete in 2 hours, when a 5 per cent suspension of washed rabbit blood cells suspended in salt solution is mixed with an equal quantity of a 24 hour broth culture. The power of the different strains to ferment the usual test substances for streptococcus has been studied. The majority fall in the group of *Streptococcus pyogenes* according to Holman's classification of streptococcus on a basis of sugar fermentations. None of the strains ferments inulin and about 20 per cent of these actively hemolytic strains possess the power of fermenting mannite. The latter characteristic will be shown to have an inter-

esting relationship to the immunological classification of these organisms.

The virulence of this group of hemolytic streptococci is low for the ordinary laboratory animals in comparison with such an organism, for instance, as pneumococcus. Doses of 1 cc. or more of a 24 hour broth culture administered intraperitoneally are required to kill guinea pigs and rabbits. Furthermore, repeated passages through these animals fail to bring about a considerable accession of virulence. The fatal dose for white rats and mice is smaller, usually in the neighborhood of 0.1 cc. of a broth culture. By continuous passage through rats and mice it has been possible to raise the virulence of a certain number of strains to a point where 0.001 cc. of broth culture is lethal for a rat in 24 hours and 0.00000001 cc. for a white mouse. On the other hand, many strains cannot be raised to this high degree of virulence even after the most persevering effort. Once the maximum of virulence is attained, this quality persists without renewed animal passages for an indefinite period of time.

The sources of the strains of *Streptococcus hæmolyticus* studied and some of their common characteristics are shown in Table I.

Although the finer differential classification of single species of bacteria by means of immune reactions is still in the earlier stages of development, enough evidence has been gathered to indicate that the more highly parasitic varieties of the species are more likely to consist of a limited number of unit types than are the less parasitic or the saprophytic members. In other words, unity of type seems to characterize the disease-producing microorganisms, whereas heterogeneity is more common among the non-pathogens. If this assumption is true it then becomes important to choose for purposes of classification the immune reactions which bring out most sharply the kind of differences sought, rather than a reaction which develops the basic relationship existing between all strains of the same species. For this purpose we regard the reactions of agglutination and protection as of superior usefulness to those of precipitation and complement fixation. The validity of any final classification arrived at depends, of course, upon the possibility of fitting accurately into such a classification a large number of strains freshly obtained from their natural environment.

TABLE I.

Source and Common Characters of Strains of Streptococcus hæmolyticus Studied.

Strain No.	Source.	Clinical diagnosis.	Hemolysis.	Sugar fermentations.				
				Lactose.	Salicin.	Mannite.	Inulin.	Raffinose.
		Type S 3.						
S 5	Autopsy (lung).	Bronchopneumonia following measles.	++++	+	+	−	−	−
S 29	Sputum.	" "	++++	+	+	−	−	−
S 114	"	" "	++++	+	+	−	−	−
S 118	Pleural fluid.	" "	++++	+	+	−	−	−
S 124	Sputum.	" "	++++	+	+	−	−	−
S 145	Throat.	" "	++++	+	+	−	−	−
S 146	Chest fluid.	" "	++++	+	+	−	−	−
S 149	Blood.	" "	++++	+	+	−	−	−
S 151	Pleural fluid.	" "	++++	+	+	−	−	−
S 2	Autopsy (blood).	Bronchopneumonia.	++++	+	+	−	−	−
S 3	" (lung).	"	++++	+	+	−	−	−
S 14	" "	"	++++	+	+	−	−	−
S 111	Chest fluid.	"	++++	+	+	−	−	−
S 53	Throat.	Measles.	++++	+	+	−	−	−
S 64	"	"	++++	+	+	−	−	−
S 154	"	"	++++	+	+	−	−	−
S 80	"	German measles.	++++	+	+	−	−	−
S 140	"	" "	++++	+	+	−	−	−
S 11	Sputum.	Lobar pneumonia (Type I).	++++	+	+	−	−	−
S 16	"	" "	++++	+	+	−	−	−
S 31	Throat.	" "	++++	+	+	−	−	−
S 44	"	" "	++++	+	+	−	−	−
S 83	"	" "	++++	+	+	−	−	−
S 95	"	" "	++++	+	+	−	−	−
S 125	"	" "	++++	+	+	−	−	−
S 144	"	" "	++++	+	+	−	−	−
S 8	"	Pneumonia.	++++	+	+	−	−	−
S 131	"	"	++++	+	+	−	−	−
S 41	"	Incipient tuberculosis.	++++	+	+	−	−	−

TABLE I—*Continued.*

Strain No.	Source.	Clinical diagnosis.	Hemolysis.	Sugar fermentations.				
				Lactose.	Salicin.	Mannite.	Inulin.	Raffinose.
		Type S 23.						
S 39	Autopsy (lung).	Bronchopneumonia following measles.	++++	+	+	−	−	−
S 78	Pleural fluid.	" "	++++	+	+	−	−	−
S 101	" "	" "	++++	+	+	−	−	−
S 107	Sputum.	" "	++++	+	+	−	−	−
S 27	Autopsy (blood).	Bronchopneumonia.	++++	+	+	−	−	−
S 67	Blood.	"	++++	+	+	−	−	−
S 120	Autopsy (blood).	"	++++	+	+	−	−	−
S 98	Throat.	Measles.	++++	+	+	−	−	−
S 116	"	"	++++	+	+	−	−	−
S 117	"	"	++++	+	+	−	−	−
S 9	"	Lobar pneumonia.	++++	+	+	−	−	−
S 23	"	" "	++++	+	+	−	−	−
S 56	Autopsy (lung).	" "	++++	+	+	−	−	−
S 65	Sputum.	" "	++++	+	+	−	−	−
S 75	Throat.	" "	++++	+	+	−	−	−
S 133	"	" "	++++	+	+	−	−	−
S 104	"	Pneumonia.	++++	+	+	−	−	−
S 130	"	"	++++	+	+	−	−	−
S 46	"	Incipient tuberculosis.	++++	+	+	−	−	−
S 122	"	" "	++++	+	+	−	−	−

TABLE I—*Continued.*

Strain No.	Source.	Clinical diagnosis.	Hemolysis.	Sugar fermentations.				
				Lactose.	Salicin.	Mannite.	Inulin.	Raffinose.
		Type S 60.						
S 137	Pleural fluid.	Bronchopneumonia following measles.	++++	+	+	+	−	−
S 6	Autopsy (lung).	Bronchopneumonia.	++++	+	+	+	−	−
S 35	Pleural fluid.	"	++++	+	+	+	−	−
S 55	Sputum.	"	++++	+	+	+	−	−
S 10	Throat.	Measles.	++++	+	+	+	−	−
S 37	"	"	++++	+	+	+	−	−
S 43	"	"	++++	+	+	+	−	−
S 60	"	"	++++	+	+	+	−	−
S 66	"	"	++++	+	+	+	−	−
S 71	"	"	++++	+	+	+	−	−
S 86	"	"	++++	+	+	+	−	−
S 88	"	"	++++	+	+	+	−	−
S 89	"	"	++++	+	+	+	−	−
S 100	"	"	++++	+	+	+	−	−
S 109	"	"	++++	+	+	+	−	−
S 123	"	"	++++	+	+	+	−	−
S 127	"	"	++++	+	+	+	−	−
S 128	"	"	++++	+	+	+	−	−
S 4	"	German measles.	++++	+	+	+	−	−
S 19	"	Lobar pneumonia.	++++	+	+	+	−	−
S 62	"	" "	++++	+	+	+	−	−
S 72	"	" "	++++	+	+	+	−	−
S 21	"	Incipient tuberculosis.	++++	+	+	+	−	−
S 141	"	" "	++++	+	+	+	−	−
S 150	"	" "	++++	+	+	+	−	−
S 267	Foot.	Cellulitis.	++++	+	+	+	−	−
S 269	Blood.	Erysipelas.	++++	+	+	+	−	−

TABLE I—*Continued.*

Strain No.	Source.	Clinical diagnosis.	Hemolysis.	Sugar fermentations.				
				Lactose.	Salicin.	Mannite.	Inulin.	Raffinose.
Type S 84.								
S 1	Autopsy (lung).	Bronchopneumonia following measles.	++++	+	+	−	−	−
S 20	" (blood).	" "	++++	+	+	−	−	−
S 110	Sputum.	" "	++++	+	+	−	−	−
S 138	"	" "	++++	+	+	−	−	−
S 50	Pleural fluid.	Bronchopneumonia.	++++	+	+	−	−	−
S 84	" "	"	++++	+	+	−	−	−
S 139	Throat.	"	++++	+	+	−	−	−
S 115	"	German measles.	++++	+	+	−	−	−
S 15	"	Lobar pneumonia.	++++	+	+	−	−	−
Unclassified.								
S 32	Autopsy (lung).	Bronchopneumonia following measles.	++++	+	+	−	−	−
S 59	" (blood).	" "	++++	+	+	−	−	−
S 93	Sputum.	" "	++++	+	+	−	−	−
S 97	Pleural fluid.	" "	++++	+	+	−	−	−
S 136	Autopsy (lung).	" "	++++	+	+	−	−	−
S 142	Sputum.	" "	++++	+	+	−	−	−
S 49	"	Bronchopneumonia following German measles.	++++	+	+	−	−	−
S 24	Autopsy (lung).	Bronchopneumonia.	++++	+	+	−	−	−
S 18	Throat.	Measles.	++++	+	+	−	−	−
S 26	"	"	++++	+	+	−	−	−
S 42	"	"	++++	+	+	−	−	−
S 51	"	"	++	+	+	−	−	+
S 63	"	"	++++	±	+	−	−	−
S 96	"	"	++++	+	+	−	−	−
S 102	"	"	++++	+	+	−	−	−
S 106	"	"	++++	+	+	−	−	−
S 108	"	"	++++	±	+	−	−	−
S 148	"	"	++++	+	+	−	−	−

TABLE I—*Concluded.*

Strain No.	Source.	Clinical diagnosis.	Hemolysis.	Sugar fermentations.				
				Lactose.	Salicin.	Mannite.	Inulin.	Raffinose.
		Unclassified—*Concluded.*						
S 17	Throat.	German measles.	+	−	−	+	−	−
S 47	"	" "	+++	+	−	−	−	−
S 48	"	" "	++++	+	+	−	−	−
S 54	Sputum.	" "	++++	+	+	−	−	−
S 68	Throat.	" "	++++	+	+	−	−	−
S 69	Autopsy (lung).	Lobar pneumonia.	++++	+	+	−	−	−
S 87	Sputum.	" "	++++	+	+	−	−	−
S 90	Throat.	" "	++++	+	+	−	−	−
S 99	Sputum.	" "	++++	+	+	−	−	−
S 34	Throat.	Incipient tuberculosis.	++++	+	+	+	−	+
S 36	"	" "	++++	+	+	−	−	−
S 121	"	" "	++++	+	−	−	−	−
S 129	"	" "	++++	+	+	+	−	−
S 155	"	" "	++++	+	+	−	−	−
S 264	Blood.	Osteomyelitis.	++++	+	+	−	−	−
S 271	"	Septicemia.	++++	+	+	−	−	−
S 272	Pus.	Abscess (measles).	++++	+	+	−	−	−
S 273	"	Scarlet fever.	++++	+	+	−	−	−
S 276	"	Pelvic abscess.	++++	+	+	−	−	−
S 277	" (abdomen).	Peritonitis.	++++	+	+	−	−	−
S 286	Pleural fluid.	Pneumonia.	++++	+	+	−	−	−
S 288	Sputum.	"	++++	+	+	−	−	−

The Reaction of Agglutination.

Specific agglutination has been found to be one of the most serviceable immune reactions for purposes of the biological classification of bacteria. In the typhoid and pneumococcus groups, for instance, it serves to distinguish clearly the different varieties from one another. It is likewise applicable to the classification of many other microorganisms. Efforts to classify the streptococci by means of this reaction apparently have not illuminated materially the relationship of one strain to another, nor have they shown a definite relationship between certain strains and a particular pathological process. A number of explanations of this fact may be proposed. In many instances no attention has been paid to the broader groupings of streptococci as determined by hemotoxin production and the fermentation of test sugars. Also streptococcus most frequently acts as a secondary invader in the production of disease, and it is probably an unwarranted assumption to suppose that type specificity is closely related to the character of the pathological process. One of the chief obstacles to the successful carrying out of the agglutination reaction has been the tendency of all types of streptococcus to undergo spontaneous granulation, and when used for tests to exhibit the phenomenon of non-specific agglutination. As a result of this, the reactions must usually be read against a more or less granular background, making it difficult, if not impossible, to distinguish between the non-specific and the specific influences. The tendency to spontaneous clumping is occasioned by several factors, only a few of which are understood. For instance, a homogeneous suspension of a granular streptococcus can easily be prepared by washing the organism several times with distilled water, and then resuspending in the same medium. The suspension will remain homogeneous for an indefinite period of time. If sodium chloride in concentrations above 0.06 per cent is added to the suspension, granulation immediately ensues. Many other salts act in the same manner. Substances antagonistic to this salt action may be added to the medium and function even to the extent of suspending the participation of the salt in the immune reaction, so that specific agglutination may be completely inhibited. Fortunately intermediate combinations can be found in which most

strains remain diffuse and in which the salt is still able to fulfill its part in the immune reaction. The most useful substance of this kind is ordinary meat infusion broth to which 1 per cent peptone has been added.[1] In addition, if streptococci are exposed to too great acidity the tendency to granulation is increased. In order to avoid this, the reaction of the medium may be so adjusted and such quantities of balanced phosphate solutions added that during growth an acidity greater than pH 7.1 is not attained. Certain other undetermined factors cause granulation, which may be defined as a general unsuitability of the medium for growth, and these can be eliminated only by experimenting with different preparations.

Technique.

The immune sera used in the agglutination and protection tests were obtained by the immunization of rabbits, sheep, and dogs. The animals were inoculated intravenously with repeated doses of heat-killed organisms, and in most instances a certain number of doses of living organisms was given. The employment for immunization of freshly isolated unpassed human strains, or the use of the same strains after a series of animal passages, does not alter in any recognizable way the specific qualities of the serum. The agglutinin and protective titer of the sera has remained undiminished for many months after the time of bleeding.

Great care is taken in the preparation of the organisms to be used in the agglutination reaction. The broth is made from carefully selected meat, and instead of the usual sodium chloride a sufficient quantity of a balanced phosphate mixture is added to give the required salt concentration and to adjust the hydrogen ion concentration at a pH of 7.4. When *Streptococcus hæmolyticus* is grown for 24 hours in a medium to which no sugar has been added, it does not develop an acidity greater than pH 7.2, which is just above the point at which the tendency to granulation appears. The organisms are removed from the culture medium by centrifugalization and are

[1] The authors are greatly indebted to Dr. Charles Krumwiede, Jr., of the Research Laboratories of the Department of Health of the City of New York, for many helpful suggestions in this technique.

washed once or twice in broth with a pH of 7.4 prepared in the manner described above. They are then resuspended in the same medium in approximately the concentration that is attained after 24 hours growth and are ready for use in the reaction. The sera to be employed in the tests are made up to the various concentrations by dilution with the same broth used for suspending the bacteria. The reaction of each specific serum is controlled by a complete series of like dilutions of normal serum of the same animal. Equal quantities of serum dilution and bacterial suspension are added to each tube and the tubes are then placed at a temperature of 55°C. for 1 hour. A temperature of 55°C. must be accurately maintained during the entire period and the tests must be read after the lapse of 1 hour, since if they are

TABLE II.

Power of Antistreptococcus Serum, Type S 3, to Agglutinate Ten Representative Strains of the Same Type.

Strain No.	Serum.	Dilution.									
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 3	Type S 3	+±	+±	+±	+±	+±	+±	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 14	Type S 3	+±	+±	+±	+±	+±	+±	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 149	Type S 3	+±	+±	+±	+±	+±	+±	+±	+±	+±	−
	Normal.	±	±(?)	−	−	−	−	−	−	−	
S 95	Type S 3	++	++	++	++	++	++	++	++	+±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 80	Type S 3	+±	+±	+±	+±	+±	+±	+±	+±	+	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 118	Type S 3	+±	+±	+±	+±	+±	+±	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 111	Type S 3	+±	+±	+±	+±	+±	+±	+±	+±	+	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 140	Type S 3	+±	+±	+±	+±	+±	+±	+±	+±	+±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 16	Type S 3	+±	+±	+±	+±	+±	+±	+±	+±	+	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 83	Type S 3	++	++	++	++	++	++	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	

TABLE III.

Test of Cross-Agglutination Reactions of Antistreptococcus Serum, Type S 3, with Strains of Streptococcus hæmolyticus of Other Types and with Unclassified Strains.

Strain No.	Type of *S. hæmolyticus.*	Serum.	Dilution.									
			1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 3	S 3	Type S 3	+ ±	+ ±	+ ±	+ ±	+ ±	+ ±	+ ±	+	±	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 23	S 23	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 107	S 23	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 67	S 23	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 65	S 23	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 75	S 23	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 128	S 60	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 55	S 60	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 60	S 60	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 267	S 60	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 4	S 60	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 84	S 84	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 1	S 84	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 50	S 84	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 20	S 84	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 115	S 84	Type S 3	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 31	Unclassified.	Type S 3	+	+	+	+	+					–
		Normal.	–	–	–	–	–					
S 108	"	Type S 3	–	–	–	–	–	–	–			–
		Normal.	–	–	–	–	–	–	–			
S 87	"	Type S 3	–	–	–	–	–	–	–			–
		Normal.	–	–	–	–	–	–	–			
S 288	"	Type S 3	–	–	–	–	–	–	–			–
		Normal.	–	–	–	–	–	–	–			
S 42	"	Type S 3	–	–	–	–	–	–	–			–
		Normal.	–	–	–	–	–	–	–			
S 121	"	Type S 3	+	±	±	–	–	–	–			–
		Normal.	–	–	–	–	–	–	–			

allowed to continue longer, non-specific granulation occurs. If clumping develops in the broth controls or in more than the first two or three dilutions of normal serum, the reaction should be regarded as unsatisfactory and discarded. By the use of this technique, it has been possible to carry out reliable agglutination tests of various strains of *Streptococcus hæmolyticus* and to show that constant type relationships exist, and that the types are sharply differentiated from one another. The results of the application of the method to strains of streptococcus described above are shown in Tables II to IX.

TABLE IV.

Power of Antistreptococcus Serum, Type S 23, to Agglutinate Ten Representative Strains of the Same Type.

Strain No.	Serum.	Dilution.									
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 23	Type S 23	++	++	++	++	++	++	+	−	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 107	Type S 23	++	++	++	++	++	++	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 27	Type S 23	+	++	++	++	+±	+	±	−	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 39	Type S 23	+	+	+±	++	+±	+	±	−	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 56	Type S 23	+	+	+±	+±	+±	+	±	−	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 67	Type S 23	+	+	+±	+±	+	+	±	−	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 98	Type S 23	+±	++	++	++	++	+±	+±	+	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 101	Type S 23	+±	++	++	++	++	+±	+±	+	±	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 104	Type S 23	+	++	++	++	++	++	+±	+	−	−
	Normal.	−	−	−	−	−	−	−	−	−	
S 130	Type S 23	+±	+±	+±	++	++	+±	+	+	+	−
	Normal.	−	−	−	−	−	−	−	−	−	

In these tables are presented the agglutination reactions of a certain proportion of the total number of strains of *Streptococcus hæmolyticus* tested. An analysis of the results shows that in the collection of

TABLE V.

Test of Cross-Agglutination Reactions of Antistreptococcus Serum, Type S 23, with Strains of Streptococcus hæmolyticus of Other Types and with Unclassified Strains.

Strain No.	Type of *S. hæmolyticus.*	Serum.	Dilution.									
			1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 23	S 23	Type S 23	±	+	+±	+±	+±	+±	+±	+	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 95	S 3	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 149	S 3	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 80	S 3	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 146	S 3	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 125	S 3	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 55	S 60	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 60	S 60	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 4	S 60	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 19	S 60	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 6	S 60	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 20	S 84	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 50	S 84	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 84	S 84	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 115	S 84	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 1	S 84	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 15	Unclassified.	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 116	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 49	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 69	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 155	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 18	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 99	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	
S 24	"	Type S 23	–	–	–	–	–	–	–	–	–	–
		Normal.	–	–	–	–	–	–	–	–	–	

organisms studied it has been possible to detect four different types of *Streptococcus hæmolyticus*. These types have been noted as Types S 3, S 23, S 60, and S 84, from the number of the chosen representative. In addition to the type strains, there remains a residue of unclassified organisms. The summary for the total number of strains studied is given in Table X.

TABLE VI.

Power of Antistreptococcus Serum, Type S 60, to Agglutinate Ten Representative Strains of the Same Type.

Strain No.	Serum.	Dilution.									
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 60	Type S 60	++	++	++		++		+±	+±	+	−
	Normal.	−	−	−		−		−	−	−	
S 269	Type S 60	+±	+±	+±	+±	+	+	±	−		−
	Normal.	±	−	−	−	−	−	−	−		
S 267	Type S 60	++	++	++	++	+±	+±	+	+		−
	Normal.	±	−	−	−	−	−	−	−		
S 55	Type S 60	++	++	++		+±		±	±	±	−
	Normal.	−	−	−		−		−	−	−	
S 128	Type S 60	++	++	++		++		+±	+±	+	−
	Normal.	−	−	−		−		−	−	−	
S 72	Type S 60	+±	+±		+±		+±	+	+	±	−
	Normal.	±	±		−		−	−	−	−	
S 43	Type S 60	++	++		+±		+±	+±	+±	+	−
	Normal.	−	−		−		−	−	−	−	
S 123	Type S 60	++	++		++		++	+±	+±	+	−
	Normal.	−	−		−		−	−	−	−	
S 66	Type S 60	++	++		++		+±	+±	+	+	−
	Normal.	−	−		−		−	−	−	−	
S 127	Type S 60	++	++		++		++	+±	+±	+	−
	Normal.	−	−		−		−	−	−	−	

The total number of strains of *Streptococcus hæmolyticus* studied was 125. Of these, 85, or 68 per cent, are comprised in the types mentioned above, and 40, or 32 per cent, remain unclassified. Work with the unclassified strains is being continued and the indications are that a certain number of other types will be discovered. In fact, two new types have already been encountered, one comprising five

TABLE VII.

Test of Cross-Agglutination Reactions of Antistreptococcus Serum, Type S 60, with Strains of Streptococcus hæmolyticus of Other Types and with Unclassified Strains.

Strain No.	Type of *S. hæmolyticus.*	Serum.	Dilution.									
			1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 60	S 60	Type S 60	++	++	++	+±	+±	+	±	±		−
		Normal.	−	−	−	−	−	−	−	−		
S 3	S 3	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 111	S 3	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 146	S 3	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−			
S 80	S 3	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 14	S 3	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 23	S 23	Type S 60	±	−	−	−	−	−	−			−
		Normal.	±	±	−	−	−	−	−			
S 78	S 23	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 98	S 23	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 107	S 23	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 122	S 23	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 84	S 84	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 115	S 84	Type S 60	−	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 15	S 84	Type S 60	±	±	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 1	S 84	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 20	S 84	Type S 60	−	−	−	−	−	−	−			−
		Normal.	−	−	−	−	−	−	−			
S 148	Unclassified.	Type S 60	+	−	−	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 96	"	Type S 60	+	±	±	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 286	"	Type S 60	±	±		±		−	−	−		−
		Normal.	−	−		−		−	−	−		
S 47	"	Type S 60	+±	+	+	−	−	−	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 102	"	Type S 60	−	+	+	+	±	±	−	−		−
		Normal.	−	−	−	−	−	−	−	−		
S 48	"	Type S 60	±	−	−		−		−	−	−	−
		Normal.	−	−	−		−		−	−	−	
S 17	"	Type S 60	−	−		−		−	−	−	−	−
		Normal.	−	−		−		−	−	−	−	

strains and another four strains, the immune reactions of which have not as yet been completed.

The antistreptococcus sera used in the agglutination reaction were obtained in the main by the immunization of sheep, a species of animal which yields a highly specific agglutinating serum for *Streptococcus hæmolyticus*. Agglutination occurred in all the type sera in dilutions of 1 : 1,000 or higher, with the exception of Type S 84, of which the

TABLE VIII.

Power of Antistreptococcus Serum, Type S 84, to Agglutinate Eight Representative Strains of the Same Type.

Strain No.	Serum.	Dilution.									
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 84	Type S 84	+±		+±	+	+	±				−
	Normal.	−		−	−	−	−				
S 1	Type S 84	+±	+±	+±	+±	+±	±				−
	Normal.	−	−	−	−	−	−				
S 50	Type S 84	+±	+±	+±	+±	+±	+				−
	Normal.	−	−	−	−	−	−				
S 20	Type S 84	++	++	+±	+±	±	−				−
	Normal.	−	−	−	−	−	−				
S 15	Type S 84	+	+±	+±	+±	+					−
	Normal.	−	−	−	−	−					
S 115	Type S 84	+±	+±	+±	+±	±					−
	Normal.	−	−	−	−	−					
S 139	Type S 84	+±	+±	+±	+±	+					−
	Normal.	−	−	−	−	−					
S 138	Type S 84	+±	+±	+±	+±	+					−
	Normal.	−	−	−	−	−					

agglutination titer has been consistently lower, usually not above 1:320. The agglutination titer of all the type sera for each strain of the same type has been approximately equal to the titer for the organism used for purposes of immunization. There has been strikingly little cross-agglutination between serum of one type and strains belonging to another. The same lack of crossing is observed among the unclassified strains with the few exceptions in which certain of these strains have shown some degree of agglutination in the type

TABLE IX.

Test of Cross-Agglutination Reactions of Antistreptococcus Serum, Type S 84, with Strains of Streptococcus hæmolyticus of Other Types and with Unclassified Strains.

Strain No.	Type of *S. hæmolyticus*.	Serum.	Dilution.									
			1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Broth.
S 84	S 84	Type S 84	+±	+±	+±	+	+	+	+			—.
		Normal.	—	—	—	—	—	—	—			
S 3	S 3	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 14	S 3	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 80	S 3	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 146	S 3	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 95	S 3	Type S 84	±	±	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 23	S 23	Type S 84	±	±	—	—	—	—	—			—
		Normal.	±	±	—	—	—	—	—			
S 107	S 23	Type S 84	±	±	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 122	S 23	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 78	S 23	Type S 84	—	—	—	—	—	—	—			—
		Normal.	—	—	—	—	—	—	—			
S 98	S 23	Type S 84	±	—	—	—	—	—	—			—
		Normal.	±	±	—	—	—	—	—			
S 60	S 60	Type S 84	+	+	±	±	—	—	—			—
		Normal.	±	±	±	±	—	—	—			
S 128	S 60	Type S 84	+±	+	+	+	—	—	—			—
		Normal.	+	+	±	±	—	—	—			
S 19	S 60	Type S 84	+	▬	±	—	—	—	—			—
		Normal.	±	±	—	—	—	—	—			
S 141	S 60	Type S 84	+	±	±	—	—	—	—			—
		Normal.	±	±	±	—	—	—	—			
S 4	S 60	Type S 84	+	±	±	—	—	—	—			—
		Normal.	+	±	±	—	—	—	—			
S 108	Unclassified.	Type S 84	—	—	—	—	—					—
		Normal.	—	—	—	—	—					
S 97	"	Type S 84	—	—	—	—	—					—
		Normal.	—	—	—	—	—					
S 54	"	Type S 84	—	—	—	—	—					—
		Normal.	—	—	—	—	—					
S 34	"	Type S 84	—	—	—	—	—					—
		Normal.	—	—	—	—	—					
S 106	"	Type S 84	—	—	—	±	±					—
		Normal.	—	—	—	—	—					

sera, but not in sufficiently high dilutions to justify their inclusion within the types. These facts show that it is possible by a series of carefully conducted agglutination experiments to determine specific type relationships between strains of *Streptococcus hæmolyticus* and to show that the different types are immunologically distinct from one another. The clearness of the agglutination reactions presented is somewhat deceptive as to the ease and simplicity of the test. It must be remembered that *Streptococcus hæmolyticus* is notoriously

TABLE X.

Summary of Agglutination Reactions.

Type of *S. hæmolyticus*.	No. of strains.	Per cent.
S 3	29	23.2
S 23	20	16.0
S 60	27	21.6
S 84	9	7.2
Unclassified	40	32.0
Total typed	85	68.0
" strains studied	125	

variable in its reactions, and that very slight and indeterminable changes in technique frequently obliterate almost completely the specificity of the agglutination reaction. In addition, a considerable number of strains is invincibly granular under all conditions and cannot be used, and occasionally strains are encountered which may occupy intermediate positions, the exact understanding of which needs a technique for the conduction of absorption experiments.

The Reaction of Protection.

Study of the power of antistreptococcus serum to protect animals against experimental infection with this organism has given rise to a number of different points of view, both regarding its action against strains from different sources, and against the same strain before and after animal passage, and also concerning the kind and the different

effect of varying antigens used in the process of immunization. For a full discussion of these matters the reader is referred to the general articles on streptococcus and to the more important papers dealing with these particular points (6). In this work they will only be considered where they have a particular bearing upon the subject under investigation. Although in the present paper the classification of *Streptococcus hæmolyticus* by means of the agglutination reaction has been presented first, practically we have obtained our primary indications of the degree of antigenic differences between strains by means of the reaction of protection. Later each reaction has been used to confirm the information obtained by means of the other.

In the successful carrying out of protection experiments two points are of especial importance: first, the production of a serum of high potency; and second, the possibility of raising the virulence of the test strains of streptococcus to such a point that very minute doses of culture are sufficient to kill white mice in a limited period of time. We have been able to produce sera in the manner alluded to above of such potency that 0.5 cc. administered intraperitoneally is sufficient to protect a white mouse against 100,000 lethal doses of a highly virulent streptococcus. In order to produce such a serum many animals must be used, only a few of which may give the desired result. It has been possible to raise the virulence of many strains by continuous passage through white mice and rats to such a point that doses of from 0.000001 to 0.00000001 cc. of broth culture are sufficient to kill the former animals in from 24 to 48 hours. These seemingly difficult conditions must be attained in order that sufficiently long-range protective titers may be carried out to insure the reliability of the information obtained. Protection against one or two lethal doses of a series of strains of streptococcus by a monovalent serum is subject to so many interpretations that the evidence gained cannot be considered of much value in judging accurately the antigenic relationship of the different strains.

The technique observed in the protocols given below has been as follows: The potency of all sera has been titrated for the homologous strain of organism and only the sera which gave a wide range of protection have been used. For infection, virulent streptococci have been used which have been grown for approximately 18 hours in either

plain broth or ascites broth. In the inoculation of animals the technique advised by Neufeld (5) has been followed with only a minor variation. The test animals have been injected intraperitoneally with 0.5 cc. of serum 24 hours before the conduction of the experiment. Tentative trials have shown that if the serum is given simultaneously with the infecting dose, no protection results, and that to insure success the serum must be given at least 8 hours before infection. On the following day a series of virulence controls is inoculated intraperitoneally, and the serum animals are injected in the same manner with doses of cultures ranging from 0.001 to 0.00000001 cc. of broth culture. Animals surviving for a period of 5 days are considered to be adequately protected. By the use of this method, it has been possible to test the antigenic relationship of a considerable number of virulent strains of *Streptococcus hæmolyticus*, and the results of these tests are set forth in the following protocols.

Protocol 1.

In this protocol is shown the titration of the serum of a sheep immunized against Strain S 23. The culture employed for infection was an 18 hour broth culture of No. S 23, which had received eighteen passages through white rats and mice. Each mouse had received 0.5 cc. of immune serum 24 hours previous to infection.

Virulence controls.		Protective power of Serum S 23.	
Dose of culture.	Result.	Dose of culture.	Result.
cc.		*cc.*	
		0.001	S.
		0.0001	D. in 4 days.
0.00001	D.* in 24 hrs.	0.000001	S.
0.000001	" "24 "	0.0000001	"
0.0000001	" "24 "	0.0000001	"

* In the tables D. indicates died, S. survived.

Protocol 2.

In this protocol is shown the protective power of Immune Serum S 3 for two virulent strains of the homologous type, for two strains of each of the heterologous types, and for two unclassified strains. The technique was the same as that in the previous protocol.

Streptococcus No.	Type of streptococcus.	Type of serum.	Dose of culture.					
			0.001 cc.	0.0001 cc.	0.00001 cc.	0.000001 cc.	0.0000001 cc.	0.00000001 cc.
S 3.18[24]*	S 3	S 3	D. 14 hrs.	S.	S.	S.	S.	
		No serum.				D. 20 hrs.	D. 23 hrs.	
S 149.16[2]	S 3	S 3	S.	S.	S.	S.		
		No serum.		D. 24 hrs.	D. 36 hrs.	D. 24 hrs.		
S 39.3[3]	S 23	S 3	D. 16 hrs.	" 16 "	" 7 "	" 19 "		
		No serum.		" 16 "	" 16 "	" 21 "		
S 67.7[3]	S 23	S 3	D. 8 hrs.	" 8 "	" 17 "	" 17 "	D. 19 hrs.	
		No serum.				" 23 "	" 21 "	
S 128.14[3]	S 60	S 3	D. 24 hrs.	D. 24 hrs.	D. 48 hrs.	" 24 "		
		No serum.		" 24 "	" 36 "	" 36 "		
S 60.10[2]	S 60	S 3	D. 18 hrs.	" 36 "	" 36 "	" 20 "		
		No serum.		" 18 "	S.	" 24 "		
S 1.8[1]	S 84	S 3	D. 22 hrs.	" 22 "	D. 22 hrs.	" 22 "		
		No serum.		" 21 "	" 21 "	" 21 "		
S 84.17[2]	S 84	S 3		" 13 "	" 19 "	" 19 "	S.	D. 20 hrs.
		No serum.				" 18 "	D. 21 hrs.	" 60 "
S 24.25[3]	Unclassified.	S 3	D. 14 hrs.	D. 16 hrs.	D. 19 hrs.	" 31 "	" 19 "	
		No serum.				" 21 "	" 28 "	
S 266.6[2]	"	S 3	D. 16 hrs.	D. 16 hrs.	D. 19 hrs.	" 21 "		
		No serum.		" 17 "	" 21 "	" 24 "		

* The integer indicates serial number of the culture, the decimal shows the number of animal passages, and the exponent the number of transplants since the last animal passage.

Protocol 3.

In this protocol is shown the protective power of antistreptococcus serum, Type S 23, against two strains of the homologous type, against two strains of each of the heterologous types, and against two unclassified strains. The technique was the same as that employed in the previous protocols.

Streptococcus No.	Type of streptococcus.	Type of serum.	Dose of culture.				
			0.001 cc.	0.0001 cc.	0.00001 cc.	0.000001 cc.	0.0000001 cc.
S 23.18[2]	S 23	S 23	D. 11 hrs.	S.	S.	S.	
		No serum.			D. 19 hrs.	D. 24 hrs.	D. 20 hrs.
S 107.12[2]	S 23	S 23	S.	S.	" 16 "	S.	S.
		No serum.			" 24 "	D. 24 hrs.	D. 24 hrs.
S 3.21[2]	S 3	S 23	D. 18 hrs.	D. 18 hrs.	" 18 "	" 36 "	" 64 "
		No serum.			" 18 "	" 36 "	" 36 "
S 80.7[2]	S 3	S 23	D. 18 hrs.	D. 18 hrs.	" 64 "	" 96 "	S.
		No serum.			S.	" 36 "	D. 36 hrs.
S 55.22[1]	S 60	S 23	D. 20 hrs.	D. 36 hrs.	D. 20 hrs.	" 30 "	
		No serum.		" 21 "	" 22 "	" 20 "	
S 60.10[1]	S 60	S 23	D. 20 hrs.	" 20 "	" 24 "	S.	
		No serum.		" 20 "	" 20 "	D. 36 hrs.	
S 84.18[1]	S 84	S 23	D. 12 hrs.	" 20 "	" 18 "	" 36 "	D. 18 hrs.
		No serum.			" 16 "	" 20 "	S.
S 50.4[2]	S 84	S 23	D. 18 hrs.	D. 36 hrs.	" 18 "	" 18 "	D. 18 hrs.
		No serum.			" 18 "	" 36 "	" 18 "
S 276.31[2]	Unclassified.	S 23	D. 16 hrs.	D. 40 hrs.	" 66 "	" 40 "	
		No serum.		" 20 "	" 18 "	" 40 "	
S 24.31[2]	"	S 23	D. 16 hrs.	" 16 "	" 16 "		
		No serum.			" 16 "	D. 20 hrs.	

Protocol 4.

In this protocol is shown the protective power of antistreptococcus serum, Type S 60, against two strains of homologous type, against two strains of each of the heterologous types, and against two unclassified strains. The technique was the same as that employed in the previous protocols.

Streptococcus No.	Type of streptococcus.	Type of serum.	Dose of culture.				
			0.001 cc.	0.0001 cc.	0.00001 cc.	0.000001 cc.	0.0000001 cc.
S 60.10[2]	S 60	S 60	D. 19 hrs.	S.	S.	S.	
		No serum.			D. 17 hrs.	D. 19 hrs.	
S 55.22[2]	S 60	S 60	S.	S.	S.	S.	
		No serum.			D. 23 hrs.	D. 16 hrs.	
S 3.22[2]	S 3	S 60	D. 18 hrs.	D. 18 hrs.	" 18 "	" 30 "	D. 24 hrs.
		No serum.			" 18 "	" 18 "	" 18 "
S 80.8[2]	S 3	S 60	D. 18 hrs.	D. 18 hrs.	" 18 "	" 22 "	" 24 "
		No serum.			" 18 "	" 18 "	" 18 "
S 75.6[1]	S 23	S 60	D. 18 hrs.	D. 21 hrs.	" 20 "		
		No serum.		" 23 "	" 17 "	D. 23 hrs.	
S 23.19[2]	S 23	S 60	D. 18 hrs.	" 18 "	" 18 "	" 24 "	D. 60 hrs.
		No serum.			" 18 "	" 60 "	" 18 "
S 84.12[1]	S 84	S 60	D. 18 hrs.	D. 18 hrs.	" 18 "	" 22 "	
		No serum.		" 18 "	" 18 "	" 20 "	
S 50.5[2]	S 84	S 60	D. 18 hrs.	" 18 "	" 18 "	" 18 "	D. 18 hrs.
		No serum.			" 18 "	" 18 "	" 18 "
S 24.28[2]	Unclassified.	S 60	D. 17 hrs.	D. 17 hrs.	" 17 "	" 24 "	
		No serum.			" 17 "	" 65 "	D. 31 hrs.
S 276.31[2]	"	S 60	D. 9 hrs.	D. 20 hrs.	" 24 "	" 41 "	
		No serum.		" 23 "	" 48 "	" 33 "	

Protocol 5.

In this protocol is shown the protective power of antistreptococcus serum, Type S 84, against two strains of the homologous type, against two strains of each of the heterologous types, and against two unclassified strains. The technique was the same as that employed in previous protocols.

Streptococcus No.	Type of streptococcus.	Type of serum.	Dose of culture.				
			0.001 cc.	0.0001 cc.	0.00001 cc.	0.000001 cc.	0.0000001 cc.
S 84.16[1]	S 84	S 84	S.	S.	S.	S.	
		No serum.		D. 16 hrs.	D. 26 hrs.	D. 54 hrs.	
S 20.5[1]	S 84	S 84	S.	S.	S.	S.	
		No serum.		D. 30 hrs.	D. 55 hrs.	D. 55 hrs.	
S 3.16[1]	S 3	S 84	D. 17 hrs.	" 17 "	" 20 "	" 29 "	
		No serum.		" 17 "	" 20 "	" 29 "	
S 14.24[1]	S 3	S 84	D. 16 hrs.	" 16 "	" 16 "	" 16 "	D. 16 hrs.
		No serum.				" 16 "	" 40 "
S 23.16[1]	S 23	S 84	D. 18 hrs.	D. 18 hrs.	D. 33 hrs.	" 32 "	
		No serum.		S.	" 22 "	" 23 "	
S 107.7[1]	S 23	S 84	D. 18 hrs.	D. 18 hrs.	" 24 "	S.	
		No serum.			" 18 "	D. 18 hrs.	D. 24 hrs.
S 128.14[2]	S 60	S 84	D. 24 hrs.	D. 24 hrs.	" 24 "	" 24 "	
		No serum.			" 24 "	" 36 "	
S 60.10[2]	S 60	S 84	D. 18 hrs.	D. 18 hrs.	" 18 "	" 21 "	
		No serum.		" 18 "	S.	" 24 "	
S 152.5[1]	Unclassified.	S 84	D. 20 hrs.	" 29 "	D. 29 hrs.	" 76 "	
		No serum.		" 16 "	S.	" 28 "	
S 266.5[3]	"	S 84	D. 15 hrs.	" 34 "	D. 23 hrs.	" 45 "	
		No serum.		" 18 "	" 38 "	" 21 "	

Consideration of the above protocols shows that the type relationships manifest from the agglutination reactions have been substantiated by the evidence obtained from the protection tests. As a matter of fact, each reaction has been used to supplement the other, the first clue as to the position of an organism sometimes being obtained by protection and sometimes by agglutination. On the whole, we are inclined to place greater confidence in the reaction of protection than in that of agglutination, and would be slow to draw general conclusions concerning type specificity from agglutination alone with such a variable organism as streptococcus, unless the results of this test could be confirmed by some other specific reaction such as protection. The sera prepared, as is seen from the protocols, have afforded a high degree of protection to white mice against infection with organisms of the homologous type. Little or no protection results when serum of one type is employed against organisms of heterologous types. There are, of course, some exceptions to this general rule. Occasionally strains of *Streptococcus hæmolyticus* are encountered against which all type sera afford a varying degree of protection, and sometimes a serum is obtained from one strain which will protect against an organism of another type, and when the reaction is reciprocally reversed no protection results. At present our knowledge is insufficient to discuss these intermediate reactions intelligently, and their elucidation must await further development of the technique. In all it has been possible to raise the virulence of 31 strains to a point where protection experiments could be performed. Of these, 7 belonged to Type S 3, 8 to Type S 23, 6 to Type S 60, 7 to Type S 84, and 3 to the unclassified group. In view of the difficulty of raising the virulence of the organisms it has been found advantageous to perform the reaction in two ways: first, to test a single monovalent serum against a number of strains; and second, to test a number of sera prepared from strains of the same type against a single virulent strain of that type. In Tables XI to XIV is shown a summary of the total number of protection experiments performed.

TABLE XI.

Summary of the Protective Power of Antistreptococcus Serum, Type S 3, against Strains of the Homologous and Heterologous Types.

Type of serum.	Strain of *S. hæmolyticus* used for production of serum.	Strain of *S. hæmolyticus* and type used for infection of mice.	Minimal lethal dose of *S. hæmolyticus* used for infection.	Protective power of 0.5 cc. of serum.
			cc.	
S 3	S 3 (Rabbit 1).	S 3 (Type S 3).	0.00000001	S. 0.0001 cc.
S 3	S 3 (" 1).	S 14 (" S 3).	0.000001	" 0.001 "
S 3	S 3 (Dog 1).	S 3 (" S 3).	0.00000001	" 0.001 "
S 3	S 3 (" 1).	S 95 (" S 3).	0.00001	" 0.01 "
S 3	S 3 (" 1).	S 80 (" S 3).	0.000001	" 0.001 "
S 3	S 3 (" 1).	S 149 (" S 3).	0.000001	" 0.01 "
S 3	S 3 (" 1).	S 146 (" S 3).	0.000001	" 0.01 "
S 3	S 3 (" 1).	S 144 (" S 3).	0.00001	" 0.001 "
S 3	S 111 (Rabbit 2).	S 3 (" S 3).	0.0000001	" 0.001 "
S 3	S 118 (" 3).	S 3 (" S 3).	0.0000001	" 0.0001 "
S 3	S 2 (" 4).	S 3 (" S 3).	0.0000001	" 0.001 "
S 3	S 11 (" 5).	S 3 (" S 3).	0.0000001	" 0.001 "
S 3	S 29 (" 6).	S 3 (" S 3).	0.00000001	" 0.0001 "
S 3	S 16 (" 7).	S 3 (" S 3).	0.00000001	" 0.0001 "
S 3	S 3 (" 1).	S 107 (" S 23).	0.0000001	D. 0.0000001 "
S 3	S 3 (" 1).	S 23 (" S 23).	0.0000001	" 0.0000001 "
S 3	S 3 (Dog 1).	S 27 (" S 23).	0.000001	" 0.000001 "
S 3	S 3 (" 1).	S 67 (" S 23).	0.00000001	" 0.0000001 "
S 3	S 3 (" 1).	S 39 (" S 23).	0.000001	" 0.000001 "
S 3	S 3 (" 1).	S 75 (" S 23).	0.000001	" 0.00001 "
S 3	S 3 (" 2).	S 56 (" S 23).	0.000001	" 0.000001 "
S 3	S 3 (" 1).	S 128 (" S 60).	0.000001	" 0.000001 "
S 3	S 3 (" 1).	S 60 (" S 60).	0.000001	" 0.000001 "
S 3	S 3 (Rabbit 1).	S 84 (" S 84).	0.00000001	" 0.00000001 "
S 3	S 3 (" 1).	S 1 (" S 84).	0.000001	" 0.000001 "
S 3	S 3 (" 1).	S 24 (unclassified).	0.00000001	" 0.00000001 "
S 3	S 3 (" 1).	S 276 (").	0.0000001	S. 0.0000001 "
S 3	S 3 (" 1).	S 61 (").	0.00001	" 0.00001 "
S 3	S 3 (Dog 1).	S 152 (").	0.000001	D. 0.000001 "
S 3	S 3 (" 1).	S 266 (").	0.000001	" 0.000001 "
Unclassified.	S 24 (Rabbit 8).	S 3 (Type S 3).	0.000001	" 0.000001 "
"	S 24 (Sheep 2).	S 14 (" S 3).	0.00000001	S. 0.00001 "
"	S 276 (Rabbit 9).	S 14 (" S 3).	0.000001	" 0.000001 "

TABLE XII.

Summary of the Protective Power of Antistreptococcus Serum, Type S 23, against Strains of the Homologous and Heterologous Types.

Type of serum.	Strain of *S. hæmolyticus* used for production of serum.	Strain of *S. hæmolyticus* and type used for infection of mice.	Minimal lethal dose of *S. hæmolyticus* used for infection.	Protective power of 0.5 cc. of serum.
			cc.	
S 23	S 23 (Sheep 1).	S 23 (Type S 23).	0.0000001	S. 0.001 cc.
S 23	S 23 (" 1).	S 107 (" S 23).	0.0000001	" 0.001 "
S 23	S 23 (" 1).	S 27 (" S 23).	0.000001	" 0.0001 "
S 23	S 23 (" 1).	S 75 (" S 23).	0.0000001	" 0.001 "
S 23	S 23 (" 1).	S 65 (" S 23).	0.0000001	" 0.001 "
S 23	S 23 (" 1).	S 3 (" S 3).	0.0000001	D. 0.000001 "
S 23	S 23 (" 1).	S 80 (" S 3).	0.0000001	S. 0.0000001 "
S 23	S 23 (" 1).	S 55 (" S 60).	0.000001	D. 0.000001 "
S 23	S 23 (" 1).	S 60 (" S 60).	0.000001	S. 0.000001 "
S 23	S 23 (" 1).	S 128 (" S 60).	0.000001	D. 0.000001 "
S 23	S 23 (" 1).	S 50 (" S 84).	0.0000001	" 0.0000001 "
S 23	S 23 (" 1).	S 84 (" S 84).	0.000001	" 0.0000001 "
S 23	S 23 (" 1).	S 24 (unclassified).	0.000001	" 0.00001 "
S 23	S 23 (" 1).	S 276 (").	0.000001	" 0.000001 "
Unclassified.	S 24 (" 2).	S 27 (Type S 23).	0.000001	" 0.000001 "
"	S 24 (" 2).	S 56 (" S 23).	0.000001	" 0.000001 "
"	S 24 (" 2).	S 107 (" S 23).	0.000001	" 0.000001 "
"	S 24 (" 2).	S 39 (" S 23).	0.00000001	" 0.000001 "
"	S 24 (" 2).	S 23 (" S 23).	0.00000001	" 0.0000001 "
"	S 276 (Rabbit 9).	S 27 (" S 23).	0.000001	" 0.000001 "
"	S 276 (" 9).	S 56 (" S 23).	0.00001	" 0.000001 "
"	S 276 (" 9).	S 107 (" S 23).	0.0000001	" 0.00000001 "
"	S 276 (" 9).	S 39 (" S 23).	0.000001	" 0.000001 "
"	S 276 (" 9).	S 23 (" S 23).	0.00000001	" 0.00000001 "
"	S 276 (" 9).	S 67 (" S 23).	0.000001	" 0.000001 "

TABLE XIII.

Summary of the Protective Power of Antistreptococcus Serum, Type S 60, against Strains of the Homologous and Heterologous Types.

Type of serum.	Strain of *S. hæmolyticus* used for production of serum.	Strain of *S. hæmolyticus* and type used for infection of mice.	Minimal lethal dose of *S. hæmolyticus* used for infection.	Protective power of 0.5 cc. of serum.
			cc.	
S 60	S 128 (Rabbit 10).	S 128 (Type S 60).	0.00001	S. 0.01 cc.
S 60	S 128 (" 10).	S 60 (" S 60).	0.000001	" 0.0001 "
S 60	S 128 (" 10).	S 55 (" S 60).	0.000001	" 0.001 "
S 60	S 128 (" 10).	S 4 (" S 60).	0.000001	" 0.001 "
S 60	S 128 (" 10).	S 72 (" S 60).	0.00001	" 0.001 "
S 60	S 128 (" 10).	S 267 (" S 60).	0.000001	" 0.001 "
S 60	S 128 (" 10).	S 3 (" S 3).	0.0000001	D. 0.0000001 "
S 60	S 128 (" 10).	S 80 (" S 3).	0.0000001	" 0.0000001 "
S 60	S 128 (" 10).	S 75 (" S 23).	0.000001	" 0.00001 "
S 60	S 128 (" 10).	S 65 (" S 23).	0.000001	S. 0.00001 "
S 60	S 128 (" 10).	S 23 (" S 23).	0.0000001	D. 0.0000001 "
S 60	S 128 (" 10).	S 84 (" S 84).	0.000001	" 0.000001 "
S 60	S 128 (" 10).	S 50 (" S 84).	0.0000001	" 0.0000001 "
S 60	S 128 (" 10).	S 24 (unclassified).	0.0000001	" 0.000001 "
S 60	S 128 (" 10).	S 276 (").	0.000001	" 0.000001 "
Unclassified.	S 24 (" 8).	S 128 (Type S 60).	0.000001	S. 0.001 "
"	S 276 (" 9).	S 128 (" S 60).	0.000001	" 0.0001 "

TABLE XIV.

Summary of the Protective Power of Antistreptococcus Serum, Type S 84, against Strains of the Homologous and Heterologous Types.

Type of serum.	Strain of *S. hæmolyticus* used for production of serum.	Strain of *S. hæmolyticus* and type used for infection of mice.	Minimal lethal dose of *S. hæmolyticus* used for infection.	Protective power of 0.5 cc. of serum.
			cc.	
S 84	S 84 (Sheep 3).	S 84 (Type S 84).	0.000001	S. 0.001 cc.
S 84	S 84 (" 3).	S 1 (" S 84).	0.000001	" 0.0005 "
S 84	S 84 (" 3).	S 20 (" S 84).	0.000001	" 0.001 "
S 84	S 84 (" 3).	S 50 (" S 84).	0.000001	" 0.0005 "
S 84	S 84 (" 3).	S 139 (" S 84).	0.000001	" 0.001 "
S 84	S 84 (" 3).	S 110 (" S 84).	0.0000001	" 0.001 "
S 84	S 84 (" 3).	S 15 (" S 84).	0.0000001	" 0.001 "
S 84	S 1 (Rabbit 11).	S 1 (" S 84).	0.00000001	" 0.00001 "
S 84	S 1 (" 11).	S 84 (" S 84).	0.00000001	" 0.0001 "
S 84	S 1 (" 11).	S 20 (" S 84).	0.000001	" 0.001 "
S 84	S 84 (Sheep 3).	S 3 (" S 3).	0.000001	D. 0.000001 "
S 84	S 84 (" 3).	S 14 (" S 3).	0.00000001	S. 0.000001 "
S 84	S 84 (" 3).	S 23 (" S 23).	0.0000001	D. 0.0000001 "
S 84	S 84 (" 3).	S 107 (" S 23).	0.0000001	S. 0.0000001 "
S 84	S 84 (" 3).	S 39 (" S 23).	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 67 (" S 23).	0.000001	D. 0.000001 "
S 84	S 84 (" 3).	S 27 (" S 23).	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 56 (" S 23).	0.00001	" 0.000001 "
S 84	S 1 (Rabbit 11).	S 107 (" S 23).	0.0000001	S. 0.00000001 "
S 84	S 1 (" 11).	S 128 (" S 60).	0.000001	D. 0.000001 "
S 84	S 84 (Sheep 3).	S 128 (" S 60).	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 60 (" S 60).	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 276 (unclassified).	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 277 (").	0.000001	S. 0.000001 "
S 84	S 84 (" 3).	S 152 (").	0.000001	D. 0.000001 "
S 84	S 84 (" 3).	S 266 (").	0.000001	" 0.000001 "
S 84	S 84 (" 3).	S 24 (").	0.000001	" 0.000001 "
Unclassified.	S 24 (Rabbit 8).	S 84 (Type S 84).	0.00001	" 0.000001 "
"	S 24 (Sheep 2).	S 1 (" S 84).	0.000001	" 0.000001 "
"	S 276 (Rabbit 9).	S 84 (" S 84).	0.00000001	" 0.00000001 "
"	S 276 (" 9).	S 20 (" S 84).	0.000001	" 0.000001 "
"	S 276 (" 9).	S 50 (" S 84).	0.000001	" 0.000001 "

DISCUSSION.

The complete biological classification of any pathogenic microorganism presents a very complex problem. The first phase of the undertaking concerns itself with the development of reliable methods for the determination of antigenic differences between members of the species and the application of these methods to the discovery of the immunological relationships between a limited number of strains purposefully selected. In this way the degree of similarity and diversity of type is shown and also the probable number of types, and the proportion of classifiable to unclassifiable strains. The next step of necessity is the testing of the adequacy and universality of the information so gained by applying the tentative classification to a large number of strains of the organism obtained under what may be described as normal conditions of pathogenicity. That some sort of equilibrium has been established in nature among microorganisms that have produced disease over long periods of time is not unlikely. Indeed, evidence obtained from the study of pneumococci supports this view (7), although departure from the norm may occur under special conditions (8). After the relationships of the pathogens of the species to one another have been discovered, it then becomes important for purposes of epidemiological study to compare by the same methods the pathogenic with the saprophytic varieties. This task requires years for its completion and many difficulties and seemingly unexplainable phenomena are encountered. In the beginning, the broader lines of differentiation must be drawn, and divergent results discarded for the time being, since, if the original conception is correct, most of the discrepancies disappear with the advance of knowledge.

In this paper are presented the facts so far obtained in the present study of *Streptococcus hæmolyticus* in accordance with the plan outlined above. The strains were collected in a limited community during the course of what may be considered an epidemic of bronchopneumonia secondary to measles. Individuals, however, from all parts of the United States were passing rapidly through this community which was a center for primary training of the aviation service, so that a wider range of territory is represented than the im-

mediate community itself. All the strains were investigated as to their cultural reactions, bile solubility, capacity to hemolyze red blood cells and to ferment the different test sugars, and as to hydrogen ion concentration limiting their growth, and thus identified as accurately as possible as *Streptococcus hæmolyticus* of the human type.

A technique was then developed for studying the immunological reactions of agglutination and protection. By the reaction of agglutination four distinct immunological types and a certain number of unclassifiable strains have been discovered among the 125 strains studied. Individuals of the same type are closely related to one another immunologically, and the different types can be sharply distinguished one from the other. In addition to the four types, study of the reactions of which has been completed, there are in addition two other types, investigation of which is as yet incomplete. The technique of the agglutination reaction demands great care, both as regards the handling of the organisms and the preparation of the medium for their growth. In the medium used by us, a large percentage of strains has grown sufficiently diffusely to permit the preparation of stable suspensions. To what extent continuous growth in this medium has promoted the tendency to diffuseness, and whether the same percentage of freshly isolated strains will grow diffusely, we are as yet unable to say. We have found that by the immunization of sheep a highly specific agglutinating serum is obtained, but that the serum produced from rabbits is not so specific and may show a wider range of crossing, especially in one of the types of streptococcus described Variations in the specificity of different animal sera have been observed by students of the immunological reactions of meningococcus. In order fully to understand this phenomenon, it would be necessary to compare the specificity of immune sera produced from different species of animals by means of the method of absorption. It is not as yet possible to undertake this kind of an investigation of *Streptococcus hæmolyticus*. The observation has been made, however, that rabbit sera showing non-specific cross-agglutination reactions in general fail to manifest corresponding cross-protection reactions.

Whenever it has been possible to raise the animal virulence of strains of *Streptococcus hæmolyticus*, the evidence obtained from the agglutination tests has been confirmed by that gained from the pro-

tection reaction. In all instances in which this has been done, one reaction has corroborated the findings of the other. The performance of reliable protection tests has been made possible by the production of sufficiently high titer antistreptococcus sera, and by the possibility of raising the animal virulence of a certain number of strains to a high degree. The types of *Streptococcus hæmolyticus* have been noted as Types S 3, S 23, S 60, and S 84, from the serial numbers of the representative strains. This nomenclature is not put forth as a final one, since we realize that probably many other human types exist, to say nothing of the bovine and cheese varieties, and that the proportional distribution of the different varieties pathogenic for man may be very different from that represented by this work. Streptococcus is the largest of all pathogenic groups of bacteria and many years will be required to bring out the information necessary to the perfecting of an adequate classification.

It is of considerable interest that all the members of Type S 60 ferment mannite, and that none of the members of the other groups so far encountered ferments this sugar. A few unclassifiable strains, however, have been found to be mannite fermenters.

This work has cleared up a number of points about *Streptococcus hæmolyticus* which have been in dispute for many years. In the first place, *Streptococcus hæmolyticus* of human origin is not a unit type as was previously supposed, but probably consists of a number of types, at least four of which have been definitely identified. Previous investigators have stated that freshly isolated human strains change their antigenic properties on animal passage, and that the latter procedure for the development of animal virulence gives a common antigenic character to all strains. We have found no evidence to support this contention; in fact immune sera produced with human strains that have never been passed through animals afford a high degree of protection against strains that have received many animal passages. In addition, the antigenic differences between strains of *Streptococcus hæmolyticus* which have been passed through animals are as distinct as those between strains which have not been so passed. The types of *Streptococcus hæmolyticus* studied have been obtained almost exclusively from the respiratory tract and from a limited source of supply, and there is some reason to believe that those which pro-

duce cellulitis, erysipelas, and septicemia may be of somewhat different character. It is, therefore, readily seen that only a beginning has been made in the classification of *Streptococcus hæmolyticus*, and that before the classification is complete and the relative dominance of the different pathogenic varieties determined, much work must be done.

SUMMARY.

1. Immunological differences have been shown to exist between strains of *Streptococcus hæmolyticus* of the human type.

2. Four biological types have been identified by means of the reactions of agglutination and protection.

3. At least two other types have been encountered and the indications are that more exist.

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THE OXYGEN OF THE ARTERIAL AND VENOUS BLOOD IN PNEUMONIA AND ITS RELATION TO CYANOSIS.

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Plates 13 to 15.

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In the recent epidemic of influenza with its accompanying pneumonia the unusual frequency of cyanosis was striking, and was in fact one outstanding feature of the epidemic. To find the cause, if possible, of the cyanosis a group of pneumonia cases at the Hospital of The Rockefeller Institute was studied during the past winter, with particular reference to cyanosis and its relation to the arterial and venous blood oxygen.

Studies of the venous blood have been made, notably by Lundsgaard[1] in cardiac insufficiency, and Harrop[2] in pneumonia; but the interpretation of the results is difficult, because of the undeterminable factors which affect the venous oxygen, such as variations in the rates of circulation and metabolism in the parts from which the blood is drawn. It appeared that satisfactorily complete data on which to base an explanation of the cyanosis could be expected only from analyses of arterial as well as venous blood. A group of 33 cases of pneumonia is here presented in which the oxygen of both arterial and venous blood has been determined.

Method.

Technique of Arterial Puncture.—Hürter[3] has shown that puncture of the radial artery is a safe procedure. At the Hospital of

[1] Lundsgaard, C., *J. Biol. Chem.*, 1918, xxxiii, 133; *J. Exp. Med.*, 1918, xxvii, 179, 219.

[2] Harrop, G. A., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 10.

[3] Hürter, *Deutsch. Arch. klin. Med.*, 1912, cviii, 1.

The Rockefeller Institute arterial puncture has been done six times on one patient without injury. In all, about 90 punctures have been done and no ill results have been observed. The possible dangers are hemorrhage, thrombosis, embolism, or aneurysm, but in this series these have never been observed, even after 4 to 5 months observation. Occasionally if proper precautions are not observed, an undue amount of blood extravasation occurs; this has been observed here once, but the blood was rapidly absorbed and no after effects were seen.

An ordinary 20 cc. all glass Luer syringe is used with a Luer needle 1 to 2 mm. in diameter. The point of the needle is beveled at an angle of about 45° and must be very sharp. To prevent the blood from coming in contact with air, 1 or 2 cc. of sterile albolene are poured into the barrel of the syringe, the plunger is inserted, and the syringe with the attached needle inverted. The plunger is forced upward, and the air in the dead space at the distal end of the syringe and needle is expelled. The excess of albolene is then forced out so that only a small amount remains in the needle and in the small dead space. The patient's arm is laid horizontally upon a pillow, the hand is flexed backwards, and the region over the radial artery is sterilized with tincture of iodine. The end of the left index finger of the operator is then sterilized with iodine and, by using this finger in palpation, the best site for the puncture is determined. (Since the position of the artery is determined solely by palpation, it is advantageous to avoid gloves and use the bare finger.) The skin at the site of the proposed puncture is anesthetized with novocaine. The syringe and needle are held at an angle of about 45° to the surface, the needle is then pushed through the skin, and, after carefully relocating its position, the needle is entered into the artery. It is essential that the position of the artery should be sharply located and the point of maximum pulsation chosen (usually opposite the radial styloid); then the artery is easily entered, the pressure of the blood stream forces up the plunger so that suction is unnecessary, and within 15 to 60 seconds from 10 to 20 cc. of blood can be obtained. The needle is then quickly withdrawn, and by means of a compress, firm pressure is immediately applied over the artery for 1 or 2 minutes, so as to obliterate it temporarily and prevent extravasation. The

wrist is then bandaged with three or four thicknesses of compress to get greater pressure, and at the end of about 2 to 3 hours the bandage may be removed. If the artery is missed at the first puncture, and especially if a hematoma begins to form or blood extravasates around the needle, the operator should desist at once.

The blood thus collected is transferred to a tube 2.5 by 10 cm. in which a layer of albolene at least 2 cm. deep has previously been placed (to prevent contact with air) with some potassium oxalate to prevent coagulation.

Not all cases are suitable for arterial punctures, especially where repeated punctures are contemplated. Many women and some men have small radial arteries deeply situated, and hence difficult to puncture. The more rapid and bounding the pulse, the easier the puncture; and cases with pulse rates of 70 or below are difficult. Occasionally when the artery has been touched with the point of the needle it becomes almost pulseless, due no doubt to reflex vasoconstriction, but after 15 to 60 seconds it relaxes and the puncture may be finished.

Following the puncture there is a numbness of the radial side of the hand, but this wears off rapidly. About 50 per cent of the subjects complain of very slight dull pain at the wrist lasting about 12 to 24 hours, but this is without significance.

In two cases which were autopsied the radial arteries, which had been punctured several times, were dissected out. In one case the sites of the punctures were difficult to determine, and there was only a small amount of extravasation of blood. In the second case one puncture had been made 4 or 5 hours before death, when the patient was almost pulseless and the puncture difficult; here there was a moderate amount of extravasated blood in the tissues surrounding the artery, and the site of the puncture was marked by a pin-head point of extravasation of blood in the wall of the artery.

Technique of Venous Puncture.—The venous blood was obtained without stasis by the technique devised by Lundsgaard.[1]

Determination of Oxygen Content, Oxygen Capacity, and Oxygen Unsaturation.[1]—The method of Van Slyke[4] was used. The arterial

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1918, xxxiii, 127.

TABLE I.

Determination of Oxygen Content, Oxygen Capacity, and Oxygen Unsaturation.

Case No.	Day of disease.	Diagnosis and clinical notes.	Result.	Cyanosis.						Oxygen content.		Oxygen capacity per 100 cc. of blood.	Oxygen unsaturation.			
													Arterial.		Venous.	
				General.	Cheeks.	Nose.	Lips.	Ears.	Fingers.	Arterial, per 100 cc. of blood.	Venous, per 100 cc. of blood.		Per 100 cc. of blood.	Per cent.	Per 100 cc. of blood.	Per cent.
										cc.	*cc.*	*cc.*	*cc.*		*cc.*	
3	6th	Lobar pneumonia.	R.*	0†	0	0	0	0	0	18 4	14.8	20.1‡	1 7	8.5	5.3	26 4
	8th	Acutely ill.		0	0	0	0	0	0	17 7	15 0	20 1‡	2 4	11 9	5.1	25.4
	11th			2	1	0	0	0	2	17.7		20.6	2.9	14.1		
	14th	After crisis; much better.		1	1	0	0	0	1	19.1	17.5	21.0	1.9	9.0	3.5	16.7
	20th	Convalescing.		0	0	0	0	0	0	18.9	15.1	20.1	1.2	6.0	5.0	24.9
	30th	"		0	0	0	0	0	0	19.9	17.8	19.9	0.0	0.0	2.1	10.5
4	7th	Influenza; bronchopneumonia. Extremely ill.	D.	3	3	2	1	1	3	15.1	5.3	20 3	5.2	25.6	15.0	73.9
5	10th	Influenza; bronchopneumonia. Heart's blood, 5 p.m.	"	4	4	4	4	4	4		3.6	23.2			19.6	84.5
				During illness.												
8	24th	Influenza; bronchopneumonia.	R.	0	0	0	0	0	0	17.1	12.0	18.6	1.5	8.1	6.6	35.5
9	9th	Bronchopneumonia.	D.	4	4	3	1	2	4	7.9	3.6	24.8	16.9	68.2	21.2	85.5
10	18th	"	R.	0	0	0	0	0	0	22.7	18.5	23.8	1.1	4.6	5.3	22.3
11	7th	Influenza; bronchopneumonia; pleuritic effusion.	"	2	2	0	0	1	2	23.1	20.1	25.7	2.6	10.1	5.6	21.8
	22nd			0	0	0	0	0	0	21.5	16.1	23.6	2.1	8.9	7.5	31.8

14	9th	Lobar pneumonia; influenza.	D.	3	2	1	1	1	3	21.1	10.6	26.6	5.5	20.7	16.0	60.2
15	3rd	Influenza; bronchopneumonia.	R.	1	1	0	0	0	1	20.0	16.7	21.7	1.7	7.8	5.0	23.0
	11th			0	0	0	0	0	0	19.1	16.4	20.6	1.5	7.3	4.2	20.4
16	4th	Bronchopneumonia.	D.	3	3	2	0	2	3	20.7	13.8	24.1	3.4	14.1	10.3	42.8
17	13th	Lobar pneumonia.	R.	1	1	0	0	0	1	16.9	16.2	20.2	3.3	16.3	4.0	19.8
	15th			1	1	1	1	0	0	16.1	9.0	18.2	2.1	11.5	10.2	56.0
18	23rd	" "	D.	2	2	1	1	0	2	19.6	17.7	23.5	3.9	16.6	5.8	24.7
	24th	Influenza; empyema.		3	3	2	1	2	3	19.8	17.1	23.7	3.9	16 5	6 6	27.8
	26th			4	4	3	2	3	4	14.4	1.3§	23.3	8.9	38.2	22.0	94.5§
19	4th	" bronchopneumonia.	R.	0	0	0	0	0	0	21.0	17.5	22.8	1.8	7.9	5.3	23.2
	5th			0	0	0	0	0	0	21.7	17.2	23.4	1.7	7.3	6.2	26.5
	13th			0	0	0	0	0	0	20.7	17.2	20.7	0.0	0.0	3.5	16.9
20	11th	" "	"	1	1	0	1	0	1	18.1	15.7	20.9	2.8	13.4	5.2	24.9
21	7th	" "	D.	4	4	4	2	3	4	10.8	4.7	22.5	11.7	52.0	17.8	79.2
	7th	Heart puncture (p.m.; 1½ hrs.).									1.8	22.5			20.7	
22	11th	Lobar pneumonia.	"	2	2	1	1	0	2	18.6	9.3	23.1	4.5	19.5	13.8	59.8
	13th			2	2	1	1	1	2	16.7	12.7	22.3	5.6	25.1	9.6	43.0

* R. indicates recovered, D. died.

† The numbers in this column indicate plus signs; *i. e.*, 0 indicates no cyanosis, 1 indicates + (slight cyanosis), 2 indicates ++ (moderate cyanosis), 3 indicates +++ (marked cyanosis), and 4 indicates ++++ (intense cyanosis).

‡ Determined colorimetrically (Palmer method—Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119).

§ Postmortem heart's blood.

TABLE I—*Concluded.*

Case No.	Day of disease.	Diagnosis and clinical notes.	Result.	Cyanosis.						Oxygen content.		Oxygen capacity per 100 cc. of blood.	Oxygen unsaturation.			
				General.	Cheeks.	Nose.	Lips.	Ears.	Fingers.	Arterial, per 100 cc. of blood.	Venous, per 100 cc. of blood.		Arterial.		Venous.	
													Per 100 cc. of blood.	Per cent.	Per 100 cc. of blood.	Per cent.
										cc.	*cc.*	*cc.*	*cc.*		*cc.*	
23	6th	Influenza; bronchopneumonia.	R.	1	1	1	1	0	1	18.5	11.3	21.8	3.3	15.1	10.5	48.2
	7th			1	1	1	1	0	1	21.6	9.2	23.7	2.1	8.9	14.5	61.2
24	6th	" "	"	0	0	0	0	0	0	17.9	16.7	18.6	0.7	3.8	1.9	10.2
	11th			0	0	0	0	0	0	20.5	11.4	21.0	0.5	2.4	9.6	45.7
	25th			0	0	0	0	0	0	19.1	15.9	19.9	0.8	4.0	4.0	20.1
25	9th	" "	D.	3	3	2	1	2	3	15.7	12.6	28.1	12.4	44.1	15.5	55.2
29	14th	Lobar pneumonia.	R.	0	0	0	0	0	0	19.9	14.8	20.4	0.5	2.5	5.6	27.5
30	6th	Influenza; bronchopneumonia.	D.	3	3	1	2	2	3	17.2	7.9	22.9	5.7	24.9	12.0	48.3
31	4th	Multiple pulmonary abscesses; empyema.	"	2	1	1	0	1	2	17.1	15.7	20.2	3.1	15.3	4.5	22.3
	9th			1	0	0	0	0	2	17.8		19.6	1.8	9.2		
32	6th	Influenza; bronchopneumonia.	"	1	0	1	1	1	2	20.7	9.7	24.7	4.0	16.2	15.0	60.8
	7th			3	2	2	1	2	4	19.9	11.8	26.2	6.3	24.0	14.4	55.0
33	8th	" "	"	2	1	1	0	1	2	17.0	13.5	22.2	5.2	23.4	8.7	39.2
34	10th	Bronchopneumonia.	"	3	3	1	1	2	3	12.9		17.1	4.2	24.6		
	13th			3	3	2	1	2	3	11.3		16.2	4.8	29.6		

35	16th	Bronchopneumonia.	R.	3	3	1	2	2	3	12.8	10.2	19.1	6.3	33.0	8.9	46.7
	17th			2	3	1	0	1	2	13.7	9.7	17.9	4.2	23.5	8.2	45.7
	23rd			3	3	2	1	2	3	11.7		15.8	4.1	25.9		
	38th			1	0	0	0	0	1	13.3		14.9	1.6	10.7		
36	9th	"	"	1	0	0	0	0	1	22.8	17.7	26.3	3.5	13.3	5.6	21.3
37	7th	"	"	0	0	0	0	1	1	20.5		21.8	1.3	6.0		
	8th			0	0	0	0	0	0	20.4	18.8	22.0	1.6	7.3	3.2	14.4
38	4th	Lobar pneumonia.	"	0	0	0	0	0	1	17.4	12.5	19.0	1.6	8.4	6.5	34.2
39	5th	Bronchopneumonia.	"	2	2	2	2	2	2	18.3	12.9	21.4	3.1	14.5	8.5	39.7
40	7th	"	D.	3	2	2	2	1	3	16.2		22.3	6.1	27.3		
41	9th	"	"	3	2	1	1	3	3	20.7		28.6	7.9	27.6		
42	9th	"	"	4	2	2	3	2	4	8.1		17.8	9.7	54.5		

and venous samples were taken under albolene to prevent contact with air and analyzed in duplicate immediately for oxygen content. A portion of the blood was saturated with oxygen and the total oxygen capacity determined, as described by Van Slyke. Thus are obtained (1) arterial oxygen content (cubic centimeters of oxygen combined with hemoglobin in 100 cc. of arterial blood), (2) venous oxygen content (a similar value for venous blood), (3) total oxygen capacity (cubic centimeters of oxygen combined with the hemoglobin of 100 cc. of blood when fully saturated).

The difference between oxygen content and total oxygen capacity has been named by Lundsgaard[1] the oxygen unsaturation, and we have followed his usage of the term. The unsaturation may be expressed either as cubic centimeters of oxygen per 100 cc. of blood, or as percentage of the total oxygen capacity. In the latter case the data represent the per cent of total hemoglobin in the form of reduced hemoglobin. An example will make this clear.

	cc.	*per cent*
Arterial oxygen content	18.0	90.0
Venous oxygen content	14.0	70.0
Total oxygen capacity	20.0	100.0
Arterial oxygen unsaturation	2.0	10.0
Venous oxygen unsaturation	6.0	30.0

RESULTS.

Table I gives the results obtained in the 33 cases of pneumonia studied.

38 cases were studied which were divided as indicated in Table II.

TABLE II.

Classification of Cases.

Diagnosis.	No
Pneumonia, lobar	7
Postinfluenzal bronchopneumonia	25
Multiple pulmonary abscesses	1
Normal individuals	5
Total	38
Complications, empyema	3

Results in Normal Controls.—Table III gives the results obtained in five normal resting men. All these subjects were up and about, but were punctured 15 to 30 minutes after resting in bed.

The range of arterial oxygen content is from 17.9 to 22.1 cc. per 100 cc. of blood. The arterial unsaturation varies from 2.8 to 6.3 per cent. The venous unsaturation varies from 22.7 to 33 per cent. The arterial blood is usually assumed to be approximately saturated, but in the five individuals given in Table III the mean value is 95 per cent. The values found for the venous oxygen unsaturation are in close accord with those of Lundsgaard in normal individuals.

TABLE III.

Arterial and Venous Oxygen, Total Oxygen Capacity, and Arterial and Venous Oxygen Unsaturation in Five Normal Individuals.

Individual No.	Oxygen content.		Oxygen capacity per 100 cc. of blood.	Unsaturation.			
				Arterial.		Venous.	
	Arterial, per 100 cc. of blood.	Venous, per 100 cc. of blood.		Per 100 cc. of blood.	Per cent.	Per 100 cc. of blood.	Per cent.
	cc.	*cc.*	*cc.*	*cc.*		*cc.*	
1	17.9	12.8	19.1	1.2	6.3	6.3	33.0
2	21.0	16.7	21.6	0.6	2.8	4.9	22.7
3	22.1	17.2	23.3	1.2	5.2	6.1	26.2
4	20.2	15.6	21.6	1.4	6.5	6.0	27.8
5	19.5	15.4	20.3	0.8	3.9	4.9	24.1
Mean.....	20.2	15.6	21.2	1.0	5.0	5.6	26.8

Arterial and Venous Oxygen in the Pneumonia Cases.—That the arterial and venous oxygen content and unsaturation in the pneumonia cases show striking contrasts to the normal individuals is seen at once from Table IV.

TABLE IV.

Maximum and Minimum Arterial and Venous Oxygen Content and Arterial and Venous Oxygen Unsaturation in Pneumonia Cases.

Arterial content per 100 cc. of blood.		Arterial unsaturation.		Venous content per 100 cc. of blood.		Venous unsaturation.	
Maximum.	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.	Maximum.	Minimum.
cc.	*cc.*	*per cent*	*per cent*	*cc.*	*cc.*	*per cent*	*per cent*
22.9	7.9	68.2	0.0	20.1	3.6	85.5	14.4

The arterial oxygen unsaturation ranges up to 68.2 per cent as contrasted to 6.3 per cent for normal individuals. For the venous unsaturation the variations are just as marked.

Oxygen Unsaturation in Fatal and Non-Fatal Cases.—If the cases are divided according to the outcome, equally characteristic differences are obtained. Table V shows that the maximum arterial unsaturation

TABLE V.

Arterial and Venous Oxygen Unsaturation in Sixteen Non-Fatal Cases.

Case No.	Maximum* unsaturation.	
	Arterial.	Venous.
	per cent	*per cent*
3	14.1	60.1
8	8.1	35.5
10	4.6	22.3
11	10.1	31.8
15	7.8	23.0
17	16.3	56.0
19	9.8	26.5
20	13.4	24.9
23	15.1	61.2
24	4.0	45.7
29	2.5	27.5
35	33.0	46.7
36	13.3	21.3
37	7.5	14.4
38	8.4	34.2
39	14.5	39.7
Mean.....	13.9	36.3

* Where more than one determination was made the maximum observed value is given.

in the recovered cases is 33 per cent, while the mean value (13.9 per cent) is more than twice the normal mean. In one case (No. 35), however, the arterial unsaturation was 33 per cent. This patient was desperately ill and the outcome for some time appeared hopeless, but she made a remarkable recovery.

Table VI shows that the arterial unsaturation in the fatal cases is much greater, the mean value being 32 per cent. Fourteen of the

sixteen fatal cases, but only one of sixteen non-fatal cases, had an arterial unsaturation greater than 20 per cent. Hence the fatal outcome of pneumonia is usually associated with a great degree of arterial unsaturation, and the arterial oxygen unsaturation offers a valuable prognostic sign. Rarely does a patient with a value greater than 20 per cent recover (one case out of 33).

A study of the venous unsaturation shows a far less degree of uniformity, and indicates the presence of factors difficult to control,

TABLE VI.

Arterial and Venous Oxygen Unsaturation in Sixteen Fatal Cases.

Case No.	Maximum* unsaturation.	
	Arterial.	Venous.
	per cent	*per cent*
4	25.6	73.9
9	68.2	85.5
14	20.7	60.2
16	14.1	42.8
18	38.2	
21	52.0	79.2
22	25.1	59.8
25	44.1	55.2
30	24.9	48.3
31	15.3	22.3
32	24.0	60.8
33	23.4	39.2
34	29.6	
40	27.3	
41	27.6	
42	54.5	
Mean.....	32.0	57.0

* Where more than one determination was made the maximum observed value is given.

which make the determination of the venous unsaturation of less prognostic significance than the arterial. However, in the fatal cases the mean value is 20 per cent higher than in the non-fatal cases. Eight of the eleven cases, or 72 per cent, showing a venous unsaturation over 47 per cent were fatal, while thirteen out of sixteen, or 81 per cent, showing less than 47 per cent venous unsaturation recovered.

Table VII gives briefly a summary of the above discussion with the maximum and minimum arterial and venous oxygen unsaturation in the fatal and non-fatal cases.

TABLE VII.

Maximum and Minimum Arterial and Venous Oxygen Unsaturation in Fatal and Non-Fatal Cases.

Type of case.	No. of cases.	Arterial unsaturation.			Venous unsaturation.		
		Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Fatal cases.....	16	68.2	14.1	32.0	85.5	22.3	57.0
Non-fatal cases.....	16	33.0	1.6	13.9	61.2	14.4	36.3
Normal individuals..	5	6.5	2.8	5.0	33.0	22.7	26.8

Cyanosis.

Cyanosis may be caused by one of three factors or any combination of these three.

1. Disturbance of the Capillary Bed.—If there is a constriction of the arterial precapillaries, or a dilatation of the capillaries due to any cause, there is a stagnation of blood in the capillaries which, in the superficial or distal parts of the body, such as the lips, nose, ears, or fingers, gives cyanosis. For example, the cyanosis after prolonged exposure to cold is undoubtedly due to this cause. Cyanosis associated with various vasomotor paralyses, as in hemiplegia and poliomyelitis, may also be explained in this way.

2. Change in the Hemoglobin.—Certain intoxications change the hemoglobin into substances which do not contain labile oxygen, and therefore reduce the oxygen capacity of the blood. These substances are methemoglobin and sulfhemoglobin, and they are found in the blood associated with the cyanosis of acetanilide, phenacetin, potassium chlorate, or nitrobenzol poisoning.[5]

Enterogenous cyanosis due to methemoglobin or sulfhemoglobin, and cases of cyanosis in which the presence of these substances can

[5] Hammarsten, O., Text book of physiological chemistry, New York, 7th edition, 1914, 283.

be shown, and in which there is a reduction of the total oxygen capacity of the blood, would fall into this class.

It is possible that the cyanosis associated with pneumonia, more particularly in the very severe cases with a marked septicemia, may in some measure be caused by this factor. Butterfield and Peabody[6] have shown that the growth of pneumococci *in vitro* results in the formation of a substance with the optical properties of methemoglobin. Further, Peabody,[7] in rabbits in which he induced an overwhelming septicemia with pneumococci, found a rapid and marked fall in the total oxygen capacity of the blood, and also observed that the blood was of a brownish color and took up oxygen slowly. However, he was rarely able to demonstrate the presence of methemoglobin in such blood, and it may be pointed out that such overwhelming septicemias as were produced in his rabbits (direct films of the blood showed numerous organisms) are never found in man. Peabody[8] and Harrop[2] found, in a few severe cases of pneumonia, a diminution in the total oxygen capacity of the blood, but were unable to demonstrate the presence of methemoglobin in the blood.

3. Admixture Cyanosis.—The third factor in the production of cyanosis is a deficient or incomplete oxygenation of the blood as it passes through the pulmonary capillaries, or the passage of but part of the blood through the lungs (as in congenital heart disease), so that there is an abnormally low percentage of oxyhemoglobin in the peripheral circulation. Obviously the study of the arterial blood oxygen would determine this.

Degree of Cyanosis and the Oxygen Unsaturation.—Cyanosis, to a greater or less degree, was observed in 28 cases out of 33. In order to compare cyanosis in different parts from time to time the following scale was used. ± indicates very slight, + slight, ++ moderate, +++ marked, and ++++ intense cyanosis.

In the five cases (Nos. 8, 10, 19, 24, and 29) of pneumonia in which no cyanosis was observed at any time, the maximum arterial un-

[6] Butterfield, E. E., and Peabody, F. W., *J. Exp. Med.*, 1913, xvii, 587.
[7] Peabody, F. W., *J. Exp. Med.*, 1913, xviii, 1.
[8] Peabody, F. W., *J. Exp. Med.*, 1913, xviii, 7.

saturation was 8.1 per cent, slightly greater than normal (Table VIII). The mean value is 5.4 per cent, only 0.4 per cent greater than for the normal individuals. The venous unsaturations follow in the same order, but are more irregular.

In the twenty-seven cases showing cyanosis during their illness (Table IX), the difference is at once apparent. Here the variations are from 7.3 to 68.2 per cent of arterial unsaturation, and all but three of the values are above 10 per cent. The mean, 24.7 per cent, is five times greater than in normal individuals. Similarly the values

TABLE VIII.

Maximum Arterial and Venous Oxygen Unsaturation in Cases without Cyanosis.*

Case No.	Arterial unsaturation.		Venous unsaturation.	
	Per 100 cc. of blood.	Per cent.	Per 100 cc. of blood.	Per cent.
	cc.		*cc.*	
8	1.5	8.1	6.6	35.5
10	1.1	4.6	5.3	22.3
19	1.8	7.9	6.2	26.5
24	0.8	4.0	9.6	45.7
29	0.5	2.5	5.6	27.5
Mean.....	1.1	5.4	6.7	31.5

*Where more than one determination was made the maximum observed value is given.

for the venous unsaturation are higher and the mean, 44.5 per cent, is greater than in the cases without cyanosis.

Not only is there a greatly increased arterial and venous unsaturation in the cases with cyanosis, but there is a definite relation between the degree of cyanosis and the degree of unsaturation. In Table X the observations are divided into five groups according to whether there was no, slight, moderate, marked, or intense cyanosis at the time the blood was obtained. There is a gradual increase in both arterial and venous unsaturation as the associated cyanosis increases. This is strikingly brought out by Text-fig. 1 which shows as a curve the relation between unsaturation and cyanosis. The curve for the venous unsaturation parallels that for the arterial unsaturation.

TABLE IX.

Maximum Arterial and Venous Oxygen Unsaturation in Cases with Cyanosis.*

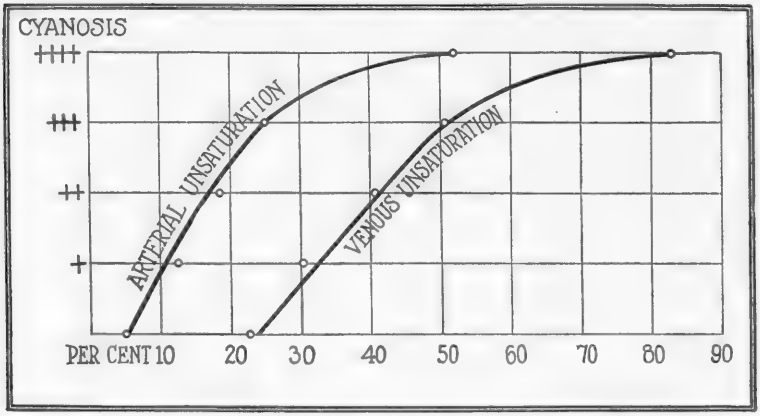
Case No.	Arterial unsaturation.		Venous unsaturation.	
	Per 100 cc. of blood.	Per cent.	Per 100 cc. of blood.	Per cent.
	cc.		*cc.*	
3	2.9	14.1	5.3	26.4
4	5.2	25.6	15.0	73.9
9	16.9	68.2	21.2	85.5
11	2.6	10.1	5.6	21.8
14	5.5	20.7	16.0	60.2
15	1.7	7.8	5.0	23.0
16	3.4	14.1	10.3	42.8
17	3.3	16.3	10.2	56.0
18	8.9	38.2	6.6	27.8
20	2.8	13.4	5.2	24.9
21	11.7	52.0	17.8	79.2
22	5.6	25.1	13.8	59.8
23	3.3	15.1	14.5	61.2
25	12.4	44.1	15.5	55.2
30	5.7	24.9	12.0	48.3
31	3.1	15.3	4.5	22.3
32	6.3	24.0	15.0	60.8
33	5.2	23.4	8.7	39.2
34	4.8	29.6		
35	6.3	33.0	8.9	46.7
36	3.5	13.3	5.6	21.3
37	1.6	7.3	3.2	14.4
38	1.6	8.4	6.5	34.2
39	3.1	14.5	8.5	39.7
40	6.1	27.3		
41	7.9	27.6		
42	9.7	54.5		
Mean.....	5.6	24.7	10.2	44.5

* Where more than one determination was made the maximum observed value is given.

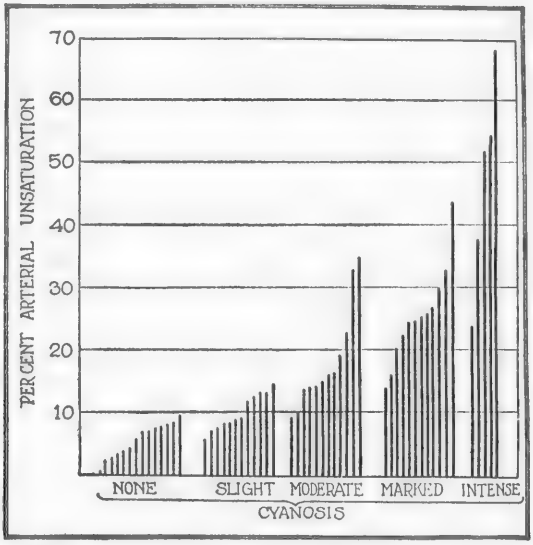
TABLE X.

Arterial and Venous Oxygen Unsaturation Associated with Cyanosis of Varying Degree.

Cyanosis.	No. of observations.	Unsaturation.	
		Mean arterial.	Mean venous.
		per cent	*per cent*
None.....	18	5.8	23.8
Slight.....	11	11.8	30.4
Moderate.....	10	17.2	41.8
Marked.....	13	26.0	51.2
Intense.....	4	53.2	82.3



TEXT-FIG. 1. Curves showing the relation between arterial and venous unsaturation and degree of cyanosis.

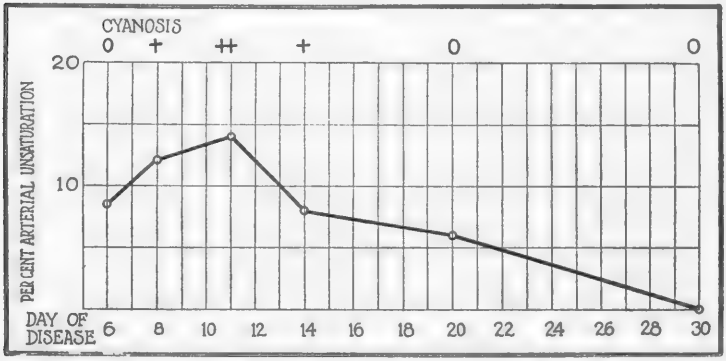


TEXT-FIG. 2. The observations of the per cent arterial unsaturation are plotted in groups according to the degree of cyanosis at the time of observation. The increase of the per cent oxygen unsaturation of the arterial blood with increasing cyanosis is striking.

In Text-fig. 2 the individual values for each observation are plotted in groups according to the degree of cyanosis. Here again the steadily increasing unsaturation with increasing cyanosis is apparent.

Again the relation of cyanosis to blood unsaturation is shown by a study of cases, in which repeated determinations were made on the same patient at different stages of the disease and with varying degrees of cyanosis.

Case 3 (Text-fig. 3) at the first observation (6th day) had no cyanosis, the arterial unsaturation being 8.5 per cent. He became much sicker and had a moderate cyanosis on the 11th day. The arterial unsaturation was then 14.1 per cent. With recovery the cyanosis disappeared, and on the 30th day the arterial unsaturation was 0.0 per cent.



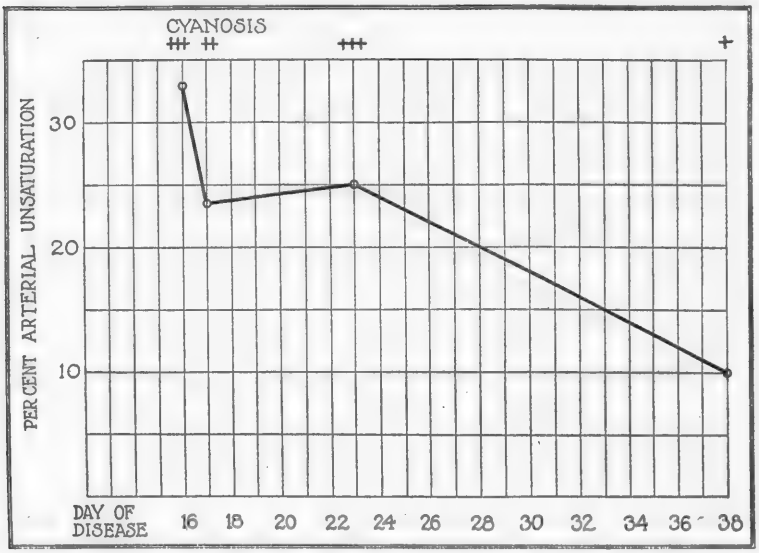
TEXT-FIG. 3. Curve of arterial oxygen unsaturation in Case 3. Note the increase in both cyanosis and arterial unsaturation as the patient became worse. After recovery the cyanosis disappeared and the arterial unsaturation became 0 per cent.

Case 35 (Text-fig. 4) was critically ill and markedly (+++) cyanotic on the 16th day; the arterial unsaturation was 33 per cent. For some time the outlook was desperate and the cyanosis continued associated with an arterial unsaturation of 23.5 and 25.9 per cent on the 17th and 23rd days. Subsequently the patient became much better; the cyanosis diminished until it was slight. The arterial unsaturation was then 10.7 per cent. After complete

recovery (95th day) when no cyanosis was present, the arterial unsaturation was 6.7 per cent.

On the other hand, Case 18 increased the arterial unsaturation from 16.6 per cent to 38.2 per cent as he became worse and the cyanosis increased (Text-fig. 5).

Discussion of Color.—The comparison of the colors of the cyanotic parts with the standard colors in "Répertoire de couleurs"[9] shows



TEXT-FIG. 4. Curve of arterial oxygen unsaturation in Case 35. There is a gradual decrease of the arterial unsaturation as the patient became better and the cyanosis diminished.

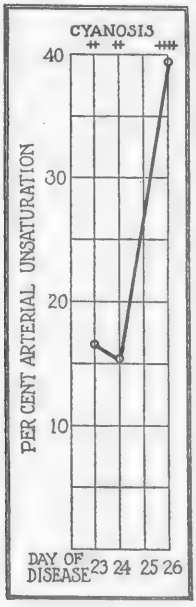
that there is considerable variation in the shades. The basic color is blue, but due to the varying admixtures with red, shades of heliotrope and mauve are frequent, especially in the fingers. On the cheeks a reddish heliotrope is not uncommon, but when the facial cyanosis is diffuse, the color is a leaden or plumbago-blue.

Distribution of Cyanosis.—The most constant and frequent site of the cyanosis was in the end of the fingers, especially under the

[9] Oberthür, R., Répertoire de couleurs, 1905.

nails. In cases with slight cyanosis a faint but definite bluish tinge could be observed here when no cyanosis could be made out elsewhere.

As the cyanosis became more intense it could next be observed over the entire end of the finger, being more marked on the dorsal aspect and fading gradually toward the first joint and toward the palmar surface.



TEXT-FIG. 5. Curve of arterial oxygen unsaturation in Case 18. The cyanosis increased from ++ to ++++ in 3 days, and the arterial unsaturation increased from 16.6 per cent to 38.2 per cent.

Fig. 1 shows the hand in Case 39 with a moderate (++) degree of cyanosis. This corresponded to 14.5 per cent of arterial unsaturation. The cyanosis is confined to the finger-nails and is of the heliotrope shade. In this case there was cyanosis of the lips, nose, and cheeks, but the color was less intense than in the finger-nails.

Fig. 2 shows the hand of a case with intense (++++) cyanosis with an arterial unsaturation of 44.1 per cent. The tips of the

fingers are a darker mauve-blue which is most intense under the nails. On the dorsum the color fades gradually beyond the terminal joint and also toward the palmar aspect.

The cyanosis of the toes is of the same order, but as a rule much less marked.

Second to the fingers the face shows cyanosis. Here it is of different types and distribution. At times even with a high degree of unsaturation, as in Case 34 (Fig. 3) with a marked (+++) degree of cyanosis, there is only a slight, dull, leaden blue, diffusely spread over the face from the forehead to and including the chin. In this case the fingers were darkly cyanotic (+++) and yet the face shows no marked accumulation of color at any one area, and seen alone would not lead one to suspect so high a degree of unsaturation.

Case 31 (Fig. 4) illustrates a second type. Here the 15.3 per cent of arterial unsaturation is accompanied by facial cyanosis in which the color is not diffuse but localized to the areas most frequently involved; namely, nose, chin, lips, ears, and cheeks.

When the cyanosis is slight it may be most marked over the malar bone. Frequently, despite a high arterial unsaturation and deep cyanosis of the fingers, the cyanosis is sharply limited to this area of the face and is of a dull cherry-red-blue at the center surrounded by a fading band of color similar to that seen in the fingers. Fig. 5 is an illustration of this type.

Even with a high arterial unsaturation the chin infrequently shows cyanosis, and the lips and ears, but occasionally. In fact the rarity and lesser degree of cyanosis of the lips in the pneumonia cases is in striking contrast to its frequency in cardiac cases.

Facial cyanosis is characterized also by its variability from day to day and from hour to hour. Often marked changes occurred within an hour. Change of position and coughing produced great changes in the intensity of the facial cyanosis. Therefore, as a measure of the degree of cyanosis from time to time the fingers are the best guide, for here the cyanosis remains most constant.

Total Oxygen Capacity.—A consideration of the oxygen capacity is important to determine whether there is methemoglobin production in pneumonia. In the entire series the total capacity varied from 14.9 to 28.6 cc. In the non-fatal cases (Table XI) there is

no unusually low capacity (except in Case 35) and the mean, 20.0 cc., is slightly lower than the normal mean. In Case 35 there was a drop in 6 days from 19.1 cc. to 15.8 cc. This patient's blood culture was negative and she was desperately ill with marked cyanosis. No examination was made for methemoglobin and the cause of this sudden drop is undetermined.

TABLE XI.

Oxygen Capacity of Non-Fatal Cases.

Case No.	Oxygen capacity per 100 cc. of blood.	Degree of cyanosis.
	cc.	
3	19.9	++
8	18.6	0
10	18.5	0
11	23.6	++
15	20.6	+
17	17.5	+
19	17.2	0
20	20.9	+
23	21.8	+
24	18.6	0
29	20.4	0
35	14.9	+++
36	26.3	+
37	21.8	+
38	19.0	+
39	21.4	++
Mean.....	20.0	

In the fatal cases (Table XII) all had marked cyanosis and should show low capacities if the formation of methemoglobin played an important part in the cyanosis. However, values even slightly below normal were shown only by Case 34. This patient was cyanotic throughout, and during a 3 day period her capacity decreased 0.9 cc. Her blood culture was sterile. With the exception of this the capacities are high and the mean, 23 cc., is higher than the normal.

Again a consideration of the changes in total oxygen capacity (Table XIII) shows that for the fatal cases there was during the illness but slight loss, and many cases show a gain. These changes

are no greater than for the non-fatal cases (Table XIV), particularly those which showed no cyanosis. Case 35 was the only case of the series which showed an unusual fall of capacity (4.2 cc.) associated with marked cyanosis.

It may be said, however, that but four cases, two of which were fatal, had positive blood cultures of pneumococci. Only one (No. 22) had an infinite number of colonies per cc. of blood; the others had but a few. This might explain why, unlike Peabody and Harrop, we failed to find occasional cases with greatly reduced oxygen capacity.

It seems unlikely, therefore, that methemoglobin formation plays any important part in the production of the cyanosis here observed, or had any part in the fatal outcome of the sixteen cases.

TABLE XII.

Oxygen Capacity of Fatal Cases.

Case No.	Time before death.	Oxygen capacity (gasometric) per 100 cc. of blood.	Degree of cyanosis.
		cc.	
4	7 hrs.	20.3	+++
5	Heart puncture (p.m.).	23.2	++++ during illness.
9	20 min.	24.8	+++
14	2 days.	26.6	+++
16	4 "	24.1	+++
18	3 "	23.7	+++
21	8 hrs.	22.5	++++
22	12 "	22.3	++
25	1 day.	28.1	+++
30	1 "	22.9	+++
31	6 days.	19.6	++
32	1 day.	26.2	+++
33	1 "	22.2	++
34	1 "	16.2	+++
40		22.3	+++
41		28.6	+++
42		17.8	++++
Mean		23.0	

A striking feature is the unusually high values for the oxygen capacities of some of the very ill or fatal cases. Cases 9, 14, 16, 25, 32, and 41 show this characteristic, but the exact cause of this high capacity is still unknown.

TABLE XIII.

Change in Oxygen Capacity in Fatal Cases.

Case No.	Change in capacity per 100 cc. of blood.	Interval.
	cc.	*days*
18	−0.2	3
22*	−0.8	2
31	−0.6	5
32	+1.7	1
34	−0.9	3

* Blood culture, Pneumococcus Type II colonies ∞ per cc.

TABLE XIV.

Change in Oxygen Capacity in Non-Fatal Cases.

Case No.	Change in capacity per 100 cc. of blood.	Interval.	Remarks.
	cc.	*days*	
3	−0.0	7	
11	−2.1	16	
15	−1.1	8	
17	−2.0	21	
19	−2.1	9	No cyanosis.
23	+2.0	1	
24	+1.3	19	No cyanosis.
35	−4.2	24	
37	+0.2	1	No cyanosis.

Oxygen Consumption and Heart Failure.

Lundsgaard[1] pointed out that the oxygen consumption, *i.e.* the difference between the arterial and venous oxygen content, increases in cardiac insufficiency. It is also increased by exercise, and presumably by other factors, such as fever, which accelerate the metabolism, unless an equivalent acceleration in circulation occurs.

The oxygen consumption in the series of pneumonia cases presented here is from 0.7 to 10.5 cc. of oxygen per 100 cc. of blood, the average values ranging from 3 to 5 cc., which is the usual range in normal individuals. As a rule, the values for the oxygen consumption in the fatal cases or in the extremely sick were no greater than those in the non-fatal cases, or in the less acutely ill. This would indicate that in the types of pneumonia (chiefly post influenza) represented by our cases the cardiac output does not fall below that normal for the resting organism.

SUMMARY.

1. A simple method for arterial puncture is given which does no permanent injury to the artery. Arterial and venous punctures have been done on 33 cases of pneumonia and five normal subjects, and the blood thus obtained has been studied with reference to the oxygen capacity and arterial and venous unsaturation.

2. In five normal subjects the mean arterial unsaturation was 5 per cent of the total oxygen capacity; the mean venous unsaturation was 26.8 per cent.

3. In the pneumonia cases the arterial oxygen unsaturation varied over a wide range. The arterial unsaturation varied from 0.0 to 68.2 per cent, the venous from 14.4 to 85.5 per cent. In the fatal cases as opposed to the non-fatal cases of pneumonia, the mean arterial oxygen unsaturation was 32 per cent as against 13.9 per cent. As a rule, an arterial unsaturation of over 20 per cent was associated with a fatal outcome. Similarly, the mean venous oxygen unsaturation was 57 per cent in the fatal cases and 36.3 per cent in the non-fatal cases.

4. In five cases in which no cyanosis was observed at any time the mean arterial oxygen unsaturation was 5.4 per cent, the mean venous oxygen unsaturation 31.5 per cent. In cases which showed cyanosis of varying degree during the course of the illness, the mean arterial unsaturation was 24.7 per cent, and the mean venous unsaturation 44.5 per cent. Cases without cyanosis have an arterial unsaturation close to the normal.

5. There is a definite relation between the degree of cyanosis and the per cent of arterial unsaturation. With increasing cyanosis

the arterial unsaturation becomes greater. The venous unsaturation varies similarly.

6. In individual cases with marked cyanosis associated with high arterial unsaturation, the clinical improvement of the patient and the diminution of the cyanosis are accompanied by a similar diminution in the arterial and venous unsaturation. Conversely, an increase of cyanosis is accompanied by an increase in arterial unsaturation.

It is evident that the cyanosis of pneumonia patients is due to the incomplete saturation of venous blood with oxygen in the lungs, and that the various shades of blue observed in the distal parts are caused by an admixture of reduced hemoglobin and oxyhemoglobin in the superficial capillaries.

7. No unusually low total oxygen capacities were observed, even in fatal cases with intense cyanosis. On the contrary, in these cases the total oxygen capacity was unusually high, pointing toward a concentration of the blood. Again in only one case was there any marked fall in the oxygen capacity during the illness. Therefore, methemoglobin formation, in these cases, can hardly have occurred to such an extent as to be an important factor in the production of cyanosis. Of the 33 cases studied, however, only seven were lobar pneumonia, the rest being of types ordinarily unusual, which have accompanied the recent influenza epidemic; and of the seven, not all were in all respects typically lobar. The possibility still remains, therefore, that in typical lobar pneumonia caused by the pneumococcus methemoglobin may play a part in the cyanosis.

8. The oxygen consumption, *i.e.* difference between arterial and venous contents, was within normal limits, indicating that the cardiac output was not diminished in the cases (chiefly post influenza) of pneumonia studied.

EXPLANATION OF PLATES.

PLATE 13.

FIG. 1. Case 39. Arterial unsaturation 14.5 per cent. There is a moderate cyanosis which is confined to the finger-nails. The color is heliotrope.

FIG. 2. Case 25. In this case there is an intense (++++) cyanosis of the fingers associated with an arterial unsaturation of 44.1 per cent. Cyanosis extends as high as the terminal joint and then fades out imperceptibly.

PLATE 14.

FIG. 3. Case 34. Bronchopneumonia; influenza. Arterial unsaturation 29.6 per cent. The entire face is diffusely cyanotic with no especial localization. The color is a leaden blue. The fingers in this case were markedly blue.

FIG. 4. Case 31. Multiple pulmonary abscesses (*Staphylococcus aureus*). Arterial unsaturation 15.3 per cent. There is a marked cyanosis of cheeks, nose, lips, and ears. On the cheek the color is a cherry-red-blue at the center, fading at the periphery to a heliotrope.

PLATE 15.

FIG. 5. Case 35. Bronchopneumonia. Arterial unsaturation 33 per cent. There is a sharply localized area of cyanosis of the cheek. The rest of the face is relatively free. The fingers had a +++ cyanosis.

FIG. 1.

FIG. 2.

(Stadie: Oxygen of the blood in pneumonia.)

FIG. 3.

FIG. 4.

(Stadie: Oxygen of the blood in pneumonia.)

FIG. 5.

(Stadie: Oxygen of the blood in pneumonia.)

THE OXYGEN AND CARBON DIOXIDE CONTENT OF ARTERIAL AND OF VENOUS BLOOD IN NORMAL INDIVIDUALS AND IN PATIENTS WITH ANEMIA AND HEART DISEASE.

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(Received for publication, May 14, 1919.)

The oxygen and carbon dioxide content of the blood in man, both in normal and in pathological conditions, has been studied but little, although the close relation of variations in the blood gases to alterations in the respiratory and circulatory mechanisms and to the blood flow has long been recognized. The difficulties of gas analysis upon small quantities of blood and in the technique of collection of samples have rendered the data hard to obtain and the reports in the literature scanty.

With the exception of a few observations by Hürter (1) upon arterial blood, all the data thus far published have been upon venous blood. Such, for example, have been the oxygen and carbon dioxide estimations of Morawitz and Röhmer (2) upon patients with anemia, those of Peabody (3) upon patients with lobar pneumonia, and those of Means and Newburgh (4) and of Lundsgaard (5) upon patients with cardiac disease.

Lundsgaard (6) has reported a series of thirty-eight determinations of the oxygen content of venous arm blood on twenty normal individuals. Hürter has reported four determinations of the oxygen and carbon dioxide content of normal human arterial blood. He found the arterial oxygen saturation to be between 93 and 100 per cent of the maximum capacity, which agrees with the values calculated from the dissociation curve for oxyhemoglobin.

The study of the arterial blood begun in 1912 by Hürter's analyses failed to develop further until 1918, when Stadie (7), continuing in the Hospital of The Rockefeller Institute the work on blood gases begun there by Lundsgaard and

Van Slyke (8), successfully undertook routine analyses of the arterial blood in patients with pneumonia.[1]

The determinations of Lundsgaard, which gave the most complete data available upon cardiac disease, were done with the recently devised blood gas apparatus of Van Slyke (8). The simplicity, accuracy, and speed attainable with this method render it a most satisfactory procedure. It has been employed in all the determinations here reported.

The collection of blood samples has been done in some instances by the method described by Lundsgaard for venous blood, and in others by the procedure recently described (9). Briefly, venous blood is collected without stasis from a vein at the bend of the elbow, and arterial blood from the radial artery, by means of a 20 cc. Luer syringe, to which is attached a sharp, short beveled needle.[2] The end of the syringe is rendered free from air by filling with 1 or 2 cc. of sterile paraffin oil, which is made to wet the barrel throughout its length. The technique of artery puncture is acquired easily, and when properly done causes little more discomfort to the patient than an ordinary venipuncture. The hand is held in the position recommended by Hürter (1), an assistant steadying it in place. After one has acquired a little practice, it is seldom necessary to use any local anesthesia. The only serious difficulty which may be met with is hematoma formation, which may take place from the opening in the artery. If proper steps to prevent it are neglected, a considerable extravasation of blood may occur and cause great pain and inconvenience. A small pressure bandage is applied tightly over the point of puncture immediately on withdrawal of the needle, and retained in place for at least an hour. Over 125 radial artery punctures and about 10 brachial artery punctures have now been done in this clinic, without any untoward effects.

Blood Gas Content in Normal Resting Individuals.

In Table I are given the results of oxygen determinations upon the arterial and venous blood of fifteen individuals with normal respiratory and circulatory apparatus, upon ten of whom simultaneous carbon dioxide analyses also were made. The artery punctures

[1] I am indebted to Dr. William C. Stadie for communicating to me his experience with the arterial puncture shortly after he had satisfied himself that it could be done without danger. In consequence of this, the present work on cardiac patients could be prosecuted simultaneously with Dr. Stadie's studies on pneumonia.

[2] The size used is No. 19 or 20 gauge, and the length 1½ to 2 inches. Care is taken that the needles are clean and very sharp.

TABLE I.

Arterial and Venous Oxygen and Carbon Dioxide Determinations upon Individuals with Normal Heart and Lung Findings.

Individual No.	Oxygen capacity (A).	Oxygen content of arterial blood (B).	Percentage saturation of arterial blood $\left(\frac{B}{A} \times 100\right)$.	Arterial oxygen unsaturation (A-B).	Oxygen content of venous blood (C).	Oxygen consumption (B-C).	Carbon dioxide content of arterial blood.	Carbon dioxide content of venous blood.	Temperature.	Pulse.	Respirations.	Blood pressure.	Remarks.
	vol. per cent	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*°F.*			*mm.*	
1	23.74	23.04	97.5	0.70	17.61	6.43	51.76	57.15	98.6	78	20	125/80	
2	17.23	17.19	100.0	None.	14.62	2.57	54.69	56.71	98.6	86	22	132/84	
3	16.26	15.31	94.3	0.95	10.52	4.79	52.89	55.88	101.4	88	22	120/80	
4	20.60	19.83	96.3	0.77	13.46	6.37	46.46	51.70	98.4	84	20	115/78	
5	18.69	17.75	95.1	0.94	15.09	2.66	44.84	48.27	99.0	84	22	130/84	
6	20.60	19.79	96.0	0.81	12.74	7.05	49.67	54.56	98.8	80	20	128/78	
7	14.42	13.89	96.3	0.53	10.77	3.12	48.13	52.18	98.8	82	20	120/80	
8	24.67	24.08	97.6	0.59	15.81	8.27	50.55	58.74	98.6	84	22	130/78	
9	18.91	17.87	94.4	1.04	13.94	3.93	53.31	60.43	98.8	86	20	126/85	
10	21.23	21.01	99.0	0.22	13.08	7.93	44.58	50.90	98.6	82	20	120/80	
11	22.02	20.94	94.9	1.08	15.04	5.90			98.4	80	24	130/85	
12	21.46	21.23	98.9	0.23	14.66	6.57			98.6	78	22	150/95	
13	20.85	19.78	94.8	1.07	14.09	5.69			98.8	78	20	128/80	
14	22.08	21.00	95.0	1.08	13.98	7.02			98.6	76	20	132/78	
15	19.76	18.92	95.8	0.84	15.17	3.75			98.6	84	22	140/80	Right radial artery.
	19.58	19.10	97.6	0.48					98.6	84	22		Left brachial artery.
	19.82	19.24	97.2	0.58					98.6	84	22		Left radial artery.

for the three determinations made on the last case in the table, No. 15, upon the right radial artery, the left radial artery, and the left brachial artery, in the order named, were all done in the space of about 12 minutes and were made with the purpose of furnishing some experimental evidence of variations, if any exist, in the blood gas content of differently placed arteries.

The term oxygen unsaturation was introduced by Lundsgaard in his studies upon the venous blood, to indicate the difference in volumes per cent between oxygen content and oxygen capacity. It is used here in the same sense.

The term oxygen consumption, used by various writers, is applied here to the difference between the oxygen content of the arterial and of the venous blood in volumes per cent.

All the blood specimens for the determinations reported in this paper were uniformly collected in the following way: The artery puncture was first done and was immediately followed by the vein puncture. Both were done within a space of 3 to 5 minutes. The pulse and respirations were then counted. The blood pressure was that recorded on the history chart as the day's reading. All the individuals were bed patients unless otherwise indicated, and the specimens were taken at least 2 hours after the preceding meal.

The determinations were done immediately after collection, in nearly all instances those for carbon dioxide by one worker and those for oxygen by the other, simultaneously. When one person has done both series, those for carbon dioxide were made first because of the tendency of the carbon dioxide to diffuse out into the protecting paraffin oil layer.

The oxygen capacity readings were in all cases checked by colorimetric determinations by the Palmer method. However, the readings taken were the average of those made directly with the blood gas apparatus.

The factors producing variation in the oxygen content of arterial blood, aside from the obvious change in the oxygen-carrying capacity of the blood due to the varying content in hemoglobin, are chiefly those due to alterations in the lung ventilation (the alveolar oxygen tension), to pathological changes in the lung tissue, and to variations due to the rate of the blood flow, as found in normal individuals, for example, between the periods of rest and exercise.

It seems unlikely that variation in the phases of normal respiration in man usually effects as large changes as have been credited to it (1 to 2 volumes per cent).

The following observation upon the oxygen content of the arterial blood in an individual with Cheyne-Stokes respirations (the period of apnea was of 45 seconds duration), certainly producing a much greater interference with the blood gas exchange in the lungs than is caused by the normal variation in the phases of respiration, offers some evidence on this point.

Specimen.	Oxygen capacity.	Oxygen content of arterial blood.	Percentage saturation of arterial blood.
	vol. per cent	*vol. per cent*	
At height of period of dyspnea.....	24.61	24.51	100 (right radial artery).
" middle " " " apnea.....		22.00	89.4 (left " ").

In spite of the marked disturbance in the breathing, the difference in the arterial oxygen content between the two phases amounted to but 2.51 volumes per cent. It was not possible to make a satisfactory collection of a venous sample, owing to the much slower rate of flow of venous blood, collected without stasis.

Little variation can take place in the gas content of the blood in the radial artery, as compared with that leaving the left heart, and there can be no changes in the particular sample due to variations in metabolism or local variations in the blood flow, which are not common to the arterial blood as a whole, as it exists in the larger vessels. The close agreement of the gas content in blood removed from various arteries (Case 15, Table I) furnishes experimental evidence on this point. The oxygen content of normal venous blood varies widely,[3] due to a combination of many factors. The most important of these are the variations in the gas content of the arterial blood and the local variations in the blood flow. The influence of the first factor is well illustrated in the determinations on patients with severe anemia, given in Table II.

The very low oxygen content of the venous blood in these patients with the resulting extremely low pressure-head in the capillaries is an indication of the ability of the tissues to take up oxygen from the

[3] According to Lundsgaard between 18 and 9.5 volumes per cent.

TABLE II.

Case No.	Date.	Oxygen capacity.	Oxygen content of arterial blood.	Arterial oxygen unsaturation.	Oxygen content of venous blood.	Oxygen consumption.	Diagnosis.
	1919	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	
1	Mar. 27	4.12	3.31	0.81	1.55	1.76	Acute lymphatic leucemia.
2	Apr. 1	4.17	3.83	0.34	1.31	2.52	Uremia. Bleeding from gums and vagina.
3	" 15	3.68	3.50	0.18	0.87	2.63	Pernicious anemia.

blood over a wide range of oxygen pressures. Even at these low levels the oxygen consumption remained within practically normal limits.

The effect upon the blood gases of alteration in the blood flow may be shown by a comparison of the analyses of blood samples taken during periods of rest and after exercise.

The experiment shown in Table III was made upon a normal individual, white, male, age 29 years. Two determinations were made (*a*) after lying down quietly for 30 minutes, and (*b*) immediately after 15 minutes of brisk exercise, consisting of arm and trunk movements, and vigorous hopping about the room until quite dyspneic. The pulse and respiration readings were then made while the arterial blood was being withdrawn. The collection of the venous blood sample was then made.

TABLE III.

Condition.	Oxygen capacity.	Oxygen content of arterial blood.	Percentage saturation of arterial blood.	Oxygen content of venous blood.	Oxygen consumption.	Carbon dioxide content of arterial blood.	Carbon dioxide content of venous blood.	Temperature.	Pulse.	Respirations.
	vol. per cent	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*°F.*		
Resting	22.04	21.09	95.6	15.08	6.01	53.33	56.90	98.6	86	18
After exercise	22.41	19.19	85.5	12.93	6.26	32.25	41.14		140	30

I hope to report at a later date a study in detail of the effects of exercise. Presumably later changes of a compensating nature may occur, in the direction of a restoration of the normal arterial oxygen saturation possibly by increased secretion of oxygen by the lung epithelium, or by regulation of the oxygen consumption in the tissues, which may explain the phenomenon of acquiring one's second wind.

Blood Gases in Cardiac Disease.

The methods just described have been applied to the study of the gas content of the blood in persons suffering from cardiac disease.[4] A considerable number of determinations has been made upon the arterial and venous blood of patients, clinically compensated, and without cardiac arrhythmias, at rest in bed. No noteworthy deviation from the values given above for normal persons has been found and it seems of no practical value to publish these results. In Table IV are given the results of determinations upon nine persons with decompensated circulatory disturbances. The clinical data upon these patients are given below.

Case 1.—J., white, male, machinist; age 50 years.

Diagnosis.—Emphysema; arteriosclerosis; hypertension; chronic myocarditis.

Previous History and Symptoms.—Shortness of breath on exertion for many years; four periods of decompensation in the past 6 years. Present attack has lasted for 3 months and has been particularly bad for the past 5 days. No history of syphilis or rheumatic fever.

Physical Examination (Apr. 25, 1919).—Slight cyanosis of finger-tips. Moderate orthopnea. Moderate engorgement of neck veins. Cardiac borders are hard to define accurately on account of the marked grade of emphysema present, but the dullness is considerably increased both to left and to right; no murmurs; no arrhythmia.. Second sounds at aortic and pulmonic areas of about equal intensity. Dullness at right lung base, with many moist râles at either base. No ascites. Pitting edema of lower legs. Wassermann reaction negative.

Apr. 28. Patient has shown rapid response to treatment. Practically no respiratory difficulty in bed. Lungs clear. Edema almost gone. Pulse regular except for one extrasystole with each 10 to 15 heart beats.

May 5. No longer any signs of myocardial insufficiency at rest in bed. Has severe attacks of cardiac dyspnea at night.

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[4] The samples were collected in these cases, as in all herein reported, upon bed patients while recumbent. The only exception was in the case of badly decompensated patients with a marked grade of orthopnea, in which instances the blood was taken from the patients when they were in a sitting posture.

TABLE IV.

Arterial and Venous Oxygen and Carbon Dioxide Determinations upon Patients with Decompensated Circulatory Disturbances.

Case No.	Age.	Date.	Oxygen capacity (A).	Oxygen content of arterial blood (B).	Percentage saturation of arterial blood $\left(\frac{B}{A} \times 100\right)$.	Arterial oxygen unsaturation (A-B).	Oxygen content of venous blood (C).	Oxygen consumption (B-C).	Carbon dioxide content of arterial blood.	Carbon dioxide content of venous blood.	Temperature.	Pulse.	Respirations.	Blood pressure.	Diagnosis.
	yrs.	*1919*	*vol. per cent*	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*°F.*			*mm.*	
1	50	Apr. 25	20.33	17.21	84.8	3.12	6.90	10.31	46.37	54.04	98.4	108	30	$\frac{138}{80}$	Emphysema; hypertension; chronic myocarditis.
		" 28	21.80	19.56	89.7	2.24	16.30	3.26	46.30	48.71	98.7	78	20	$\frac{160}{100}$	
		May 5	23.12	22.25	96.4	0.87	15.51	6.74	44.29	56.10	98.6	84	20	$\frac{160}{105}$	
2	57	Feb. 24	19.00	17.05	89.7	1.95	9.26	7.79	45.44	48.28	98.0	70	24	$\frac{140}{95}$	Emphysema; chronic myocarditis.
		" 29	18.67	17.90	95.9	0.77	9.49	8.41	48.17	52.71	98.6	72	20	$\frac{135}{90}$	
		Mar. 15	18.50	17.75	95.9	0.75	12.62	5.13	49.59	53.06	98.2	72	20	$\frac{140}{100}$	
3	50	Feb. 21	23.85	19.41	81.4	4.44	9.61	9.80			98.0	90	20	$\frac{120}{85}$	" " bronchitis; chronic myocarditis.
		" 26	23.33	20.33	87.3	3.00	12.37	7.96			98.6	84	20	$\frac{130}{85}$	
		Mar. 4	23.61	21.22	89.9	2.39	13.66	7.56			98.4	80	20	$\frac{136}{90}$	

4	60	Mar. 29	16.78	13.90	82.8	2.88	10.84	3.06	41.40	44.19	97.8	80	32	154/100	Chronic myocarditis; auricular fibrillation.
		Apr. 5	16.61	15.23	91.7	1.38	9.58	5.65	43.51	46.48	98.4	48	24	140/98	
5	30	Feb. 16	19.87	17.70	89.1	2.17	6.30	11.40	38.88	41.76	98.8	96	24	110/68	Chronic rheumatic endocarditis; mitral stenosis and insufficiency; auricular fibrillation.
		" 24	19.68	18.15	92.3	1.53	10.42	7.73	42.68	47.05	98.7	92	28	115/80	
		Mar. 4	19.74	17.68	89.5	2.06	8.31	9.37			98.4	96	28	112/78	
		" 18	19.62	17.90	89.8	1.72	13.07	4.83	43.80	47.61	98.6	88	26	116/80	
6	38	Apr. 9	18.68	16.46	88.2	2.22	12.94	3.52			98.6	140	36	110/80	Mitral and aortic insufficiency; adherent pericardium; bilateral hydrothorax.
		" 10	19.09	15.56	81.5	3.53	8.49	7.07			99.0	170	48	103/75	
7	39	Jan. 2	18.85	16.48	87.4	2.37	7.29	9.19	38.65	43.21	98.4	100	36	135	Syphilis of aorta; aortic insufficiency.
		" 10	22.92	20.52	85.9	2.40	16.10	4.42	42.87	46.48	98.6	98	26	145	
		" 20	22.48	21.09	93.7	1.39	15.42	5.67	43.62	49.01	98.4	78	20	145	
8	33	Apr. 29	20.74	19.31	93.2	1.43	11.13	8.18	31.42	37.53	98.2	96	30	128	Aortic insufficiency.
		May 1	22.50	21.30	94.7	1.20	11.64	9.66	42.80	45.70	98.0	84	24	125	
		" 6	23.25	22.49	96.8	0.76	17.55	4.94	41.47	46.52	98.6	88	24	125	
9	42	Jan. 30	17.97	16.71	93.0	1.26	6.64	10.07			97.4	100	28	140	" "
		Feb. 24	16.33	15.96	97.7	0.37	9.82	6.14			98.2	90	22	148	

It will be noticed that the percentage saturation of the arterial blood with oxygen at the time of admission was much lower than normal. The chest showed well marked emphysema, and signs of congestion in the lungs were apparent. With the clearing up of this congestion and the return to compensation the arterial oxygen saturation quickly returned to normal. At the same time the oxygen content of the venous blood rose and the oxygen consumption correspondingly became less.

Case 2.—L., negro, male, laborer; age 57 years.

Diagnosis.—Emphysema; hypertension; chronic myocarditis.

Previous History and Symptoms.—Recurrent periods of decompensation for several years. No history of syphilis or rheumatic fever.

Physical Examination (Feb. 24, 1919).—Moderate dyspnea. No definite cyanosis. Huge heart, with apex in midaxilla; no murmurs; rate regular except for an occasional extrasystole. Moderate dilatation of neck veins. Slight edema of legs. Numerous moist râles and dullness at the lung bases. Liver edge well below the costal margin and tender. Wassermann reaction negative.

Feb. 29. Patient has responded well to treatment. The lungs are clear. No liver tenderness. Practically no respiratory distress. Still some extrasystoles; no cyanosis; no edema.

Mar. 15. Condition is not changed. The extrasystoles persist.

The rapid improvement in the symptoms of decompensation in this case, which responded to treatment almost as rapidly as Case 1, produced coincident improvement in the oxygenation of the arterial blood with the clearing up of the signs of lung congestion.

Case 3.—S., white, male, occupation not given; age 50 years.

Diagnosis.—Chronic bronchitis; emphysema; chronic myocarditis.

Previous History and Symptoms.—Symptoms of emphysema and of chronic bronchitis for many years. Dyspnea on exertion for 25 years. Winter cough for past 10 years. Gradual onset more recently of the cardiac symptoms.

Physical Examination (Feb. 21, 1919).—Signs of right-sided cardiac hypertrophy, with secondary myocardial insufficiency. Voluminous, hyperresonant lungs; bases descend very little, and there are numerous fine râles. Heart borders overlapped by lungs, but distinctly enlarged to the right; the sounds are faint, almost inaudible, but seem clear. Slight edema of legs; no liver tenderness; no particular engorgement of the superficial veins. Marked cyanosis of lips, face, and finger-tips, out of proportion to the involvement of respiration. Expectoration of purulent sputum. Wassermann reaction negative.

Feb. 26. Condition is greatly improved, but there are still many râles at the lung bases, and there is considerable cyanosis.

Mar. 4. Condition further improved. Râles still present at bases. Cyanosis still present.

In this case there was distinctly evident a superimposed lung factor, long standing chronic bronchitis and emphysema, which produced a marked effect on the normal oxygenation of the blood. Even at discharge the percentage saturation of the arterial blood with oxygen was below the low normal value, and the patient was distinctly cyanotic. This chronic state of cyanosis had been present for years, although much aggravated at the time of his cardiac break. The oxygen content of the venous blood was about normal over the period of the observations.

Case 4.—M., negro, male, laborer; age 60 years.

Diagnosis.—Chronic myocarditis; auricular fibrillation.

Previous History and Symptoms.—Onset of disorder in Nov., 1918, with cough and shortness of breath. Swelling of legs 3 days ago.

Physical Examination (Mar. 29, 1919).—Marked orthopnea. Huge heart, enlarged downward and to the left; second pulmonic sound accentuated; no murmurs; rate totally irregular. Pulse deficit about 10 beats per minute. Many moist râles at either base, especially at the right. No particular cyanosis. Slight tenderness at right costal margin. Pitting edema of legs, extending up to the knees. Neck veins somewhat distended. Wassermann reaction negative.

Apr. 5. Condition much improved, although distinct orthopnea is still present. Pulse very slow, 48 per minute, all the beats now coming through to the wrist. No cyanosis or liver tenderness. Edema rapidly clearing up. Râles at lung bases have practically cleared up. Electrocardiogram indicates auricular fibrillation.

Case 5.—C., negro, female; age 30 years.

Diagnosis.—Mitral stenosis and insufficiency; auricular fibrillation.

Previous History and Symptoms.—Rheumatic fever 3 years ago. No history of syphilis. Cardiac symptoms, especially shortness of breath and occasional precordial pain, have been present during the past 10 months.

Physical Examination (Feb. 16, 1919).—Marked orthopnea and dyspnea. Totally irregular heart; about 15 beats per minute fail to come through to the wrist. Râles and dullness at the lung bases. Only moderate cyanosis of lips and finger-tips. Marked edema of legs and hands. Tender, palpable liver; certainly some fluid in abdomen. Systolic and diastolic murmurs at apex. Snapping first sound. Accentuated second pulmonic sound. Wassermann reaction negative.

Feb. 24. Rather slow response to digitalis. Condition, however, seems improved. Signs of congestion somewhat less marked.

Mar. 4. Slow but distinct improvement. Edema gone. Râles and dullness no longer present at lung bases.

Mar. 18. Seems practically compensated clinically. Heart rate 88, all beats coming through to the wrist. Lungs clear of râles.

The percentage saturation of the arterial blood in this patient tended to remain definitely below the normal figure, even after compensation was restored. As far as could be determined clinically, the lung bases were clear. There was nothing to suggest adherent pericardium; the heart, although large, was clearly movable with change in position. Nevertheless, the patient gave every indication of having had long standing cardiac disease, despite her rather short and possibly inexact history. She exhibited well marked mitral facies and clubbing of the fingers. The x-ray of the chest was reported as showing considerable infiltration, particularly of the lower parts of both lungs. The figures for the oxygen consumption tended to show the same erratic irregularities to which, in auricular fibrillation, Lundsgaard (5) has already drawn attention.

Case 6.—N., white, male, sailor; age 38 years.

Diagnosis (*Autopsy*).—Mitral and aortic insufficiency; adherent pericardium; bilateral hydrothorax.

Previous History and Symptoms.—Patient was admitted very ill. Said to have been sick 2 weeks with shortness of breath. Expectoration of blood-tinged sputum for several days.

Physical Examination (*Apr. 9, 1919*).—Marked dyspnea and cyanosis of lips, face, and fingers. Rapid but regular pulse. Lungs practically clear. Marked retrosternal dullness and huge heart. Moderate engorgement of neck vessels. No edema. Soft, pulsating liver, edge at umbilicus. Systolic and diastolic murmurs at apex and soft aortic diastolic murmur to left of sternum. Wassermann reaction negative.

Apr. 10. Condition decidedly worse, with delirium and marked cyanosis. No fever. Pulse weak and rapid. Heart and lung signs unchanged, except that signs of pulmonary edema developed rapidly shortly after the blood specimens were taken. Patient died about 16 hours later.

Autopsy.—Huge heart to which the pericardium was everywhere adherent. The lungs were clear except for the compression due to a bilateral hydrothorax of 1,500 cc. on either side. The liver was enlarged.

This case is of particular interest in that the patient showed cyanosis of extreme degree, and it was thought possible that pneumonia was present as well as the cardiac disease. This opinion proved incorrect, however, and the lung changes were all found to be secondary to the cardiac deficiency.

All the evidence pointed to a rapid appearance of serious respiratory embarrassment on account of the acute decompensation and the resulting accumulation of pleural fluid. It seems not unlikely that the time element plays a certain part in some of these cases, and that in this particular one, had the respiratory apparatus had time to adjust itself to the new conditions, a more efficient oxygen saturation of the arterial blood would have been later effected, even if the cause of the lung compression remained.

Case 7.—S., negro, male, laborer; age 35 years.

Diagnosis.—Syphilis of aorta; aortic insufficiency.

Previous History and Symptoms.—Indefinite history of shortness of breath about 1 year ago. This improved but again became serious about 6 months ago, since which time he has worked but little. Swelling of the legs and orthopnea for past 3 months. No history of rheumatism.

Physical Examination (Jan. 2, 1919).—Orthopnea; swelling of neck veins; massive pitting edema of legs; slight cyanosis. Huge heart with loud aortic diastolic murmur; rate regular. Palm's breadth of marked dullness at right base; many moist, bubbling râles at either base. Wassermann reaction positive.

Jan. 10. Condition has improved with rest and the usual cardiac treatment. Nevertheless, the pulse is 98 and there is still distinct orthopnea. Râles still persist at either base and considerable dullness is still present at the right base.

Jan. 20. Patient has gradually improved and there is no longer any respiratory distress. Pulse 78. Lung bases practically clear. No edema and no liver tenderness.

Case 8.—H., negro, male, laborer; age 33 years.

Diagnosis.—Aortic insufficiency.

Previous History and Symptoms.—Shortness of breath for 2 months. Swelling of legs noticed 2 days ago. History of syphilis. Some bloody sputum expectorated the day previous to admission.

Physical Examination (Apr. 29, 1919).—Moderate dyspnea and slight orthopnea. No cyanosis or dilatation of superficial veins. Lungs clear except for a few crackles at the bases; no dullness. Huge heart, with apex in the anterior axillary line; increased retrosternal dullness; musical aortic diastolic murmur along left sternal border and in aortic area. Collapsing pulse, regular. Very slight liver tenderness. Moderate edema of lower legs. Wassermann reaction positive.

May 1. Some bloody expectoration yesterday. Considered likely that lung infarcts have been produced, but no pain in chest is complained of, and lungs show no new findings. Breathing improved. Edema less.

May 5. Compensation is now almost regained. Lungs are clear. Pulse much slower and regular. No cyanosis; no edema; no liver tenderness.

Case 9.—R., negro, male, laborer; age 42 years.

Diagnosis.—Syphilis of aorta; aortic insufficiency.

Previous History and Symptoms.—Has had shortness of breath for several months and swelling of legs for 2 weeks. Hard chancre 4 years ago.

Physical Examination (Jan. 30, 1919).—Orthopnea and dyspnea are marked. No cyanosis. Lungs are practically clear. Wide retrosternal dullness. Huge heart with the apex impulse in midaxilla, with signs of aortic insufficiency. Typical pulse, regular in rhythm. Slight edema of legs. Tenderness at right costal margin. Some engorgement of neck vessels. Wassermann reaction positive.

Feb. 24. Compensation is quite regained. Lungs clear.

The last three patients (Cases 7, 8, and 9) exhibited the same type of cardiac lesion with varying degrees of decompensation. Of these, Case 7 was suffering from a much more severe break than the other two, and his recovery under cardiac therapy (milk diet, restricted fluids, digitalis, and rest) was much slower. His lower chest was filled with moist râles at the time of the first examination, and it seemed likely that there was a certain amount of pleural effusion at the right base.

Although it was considered probable at the time (May 1) that Patient H. (Case 8) had had a pulmonary infarct because of his bloody sputum, no other signs appeared, there was no abnormality found in the content of blood gases, and his return to compensation was prompt.

The condition of Patient R. (Case 9) at the time of the first blood oxygen examination, on the 4th day following admission, was not serious, except for his rather marked orthopnea and enlarged liver. The lungs showed no outspoken evidence of congestion. The oxygen consumption at this examination was high—presumably there was rather marked increase in the metabolism with the severe respiratory exertion, but the percentage saturation of the arterial blood was about normal. At the time of the second examination (February 24), when the findings were normal, compensation had been entirely regained.

CONCLUSION.

An examination of the data presented in these cases of cardiac disease indicates the importance of the effect of the primary condition of the lungs upon the oxygen saturation of the arterial blood during periods of decompensation. Emphysema and chronic inflammatory processes appear to aggravate greatly the effect of the passive congestion due to the cardiac insufficiency alone.

It is desired to take up at another time the effects produced by disturbances to the passage of air through the trachea and its branches. Sufficient data have accumulated, however, to warrant the expression of an opinion that the time element is of importance. Sudden mechanical disturbances to breathing produce abnormal changes in the concentration of the blood gases. These disturbances, after a period, even if unrelieved, are in large part compensated.

Because of the striking variation in the color of blood due to changes in its oxyhemoglobin content, it appears possible to relate the degree of cyanosis in individuals to the extent of oxygen unsaturation. The determining factor in the production of cyanosis must be the color of the blood in the superficial capillaries and veins, although the amount of skin pigmentation no doubt influences greatly its appreciation clinically. The cause of the phenomenon probably varies. One type is produced where incomplete saturation of the arterial blood is at fault, due to disturbance in the normal gas exchange in the pulmonary alveoli, as in pneumonia. In many cases of heart disease it may be a combination of both arterial and primarily venous unsaturation; in others it is the venous unsaturation due to stasis which is chiefly at fault. It is probable that in other instances there may be disturbance neither in the pulmonary exchange nor in the blood flow in the larger venous vessels, but that constriction of peripheral vessels due to local stimuli, with consequent local slowing of the blood flow and necessarily increased oxygen consumption, produces a type of "capillary" cyanosis, such as is, for example, strikingly seen in bathers exposed to the cold air when wet.

SUMMARY.

1. The oxygen content of venous and of arterial blood from fifteen essentially normal individuals at rest in bed has been determined.

2. The percentage saturation of the arterial blood has varied between 100 and 94.3. The average is 95.5 per cent.

3. The oxygen consumption has varied between 2.6 and 8.3 volumes per cent.

4. The oxygen content and the percentage saturation of arterial blood taken at close intervals from three different peripheral arteries of a normal individual have shown values agreeing within the limits of error. Analyses of the blood gases of a normal individual, at rest and after exercise, have shown a lowering of the percentage oxygen saturation of the arterial blood and a diminished carbon dioxide content after exercise.

5. In three persons with severe anemia the saturation of the arterial blood has not differed from the normal. Very low absolute values were found for the oxygen content of the venous blood, but the normal oxygen consumption has been maintained.

6. The carbon dioxide content of the arterial blood from ten normal individuals has varied between 54.7 and 44.6 volumes per cent. That of the venous blood has varied between 60.4 and 48.3 volumes per cent.

7. No deviations from the normal values for oxygen and carbon dioxide were found in venous and arterial blood from cardiac patients without arrhythmias, well compensated, and at rest in bed.

8. A series of determinations has been made upon nine cardiac patients with varying degrees of decompensation. The percentage oxygen saturation of the arterial blood on admission was abnormally low in seven of these cases. With the return to compensation and with the clearing up of pulmonary symptoms, the percentage saturation of the arterial blood returned to normal in four of them.

9. In a case of long standing mitral endocarditis with auricular fibrillation it remained low over a period of 1 month of observation.

10. In a case of chronic myocarditis secondary to emphysema and chronic bronchitis, it remained low over the period of observation.

11. Normal values for the percentage saturation of the arterial blood were found in two individuals with decompensated aortic disease but without physical signs of extensive pulmonary involvement.

12. The oxygen consumption tended to be high in individuals with cardiac disease during the periods of marked decompensation and to be lower as compensation was regained.

13. The data presented indicate that at least in many circulatory diseases during decompensation, particularly when there are physical signs of pulmonary congestion, there is a disturbance of the pulmonary exchange, as indicated by the lowering of the percentage saturation of the arterial blood with oxygen.

I desire to acknowledge the assistance of Miss Ethel M. Benedict, Assistant in Medicine, who performed many of the gas analyses used in this paper.

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STUDIES ON CYANOSIS.

I. Primary Causes of Cyanosis.

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INTRODUCTION.

In medical terminology cyanosis indicates a blue or bluish color of the skin of the mucous membranes, and of other organs usually not visible (the kidneys, the spleen, etc.). Cyanosis may be general or local. In the latter instances, it is usually found on the lips, nose, ears, fingers, and toes (acrocyanosis). It may be met with in various pathological conditions, and is found especially in patients suffering from diseases of the heart, lungs, and blood. Since it is a striking sign, cyanosis has been known since the early history of medicine, and has attracted much attention.

Various theories have been put forward about its pathogenesis, but none has been generally accepted. Cyanosis is an important symptom in the differential diagnosis of various diseases, and it has always been considered important for the prognosis of the diseases in which it is encountered. Since it is usually considered a harmful condition, special therapeutic procedures (venesection, oxygen inhalations) have often been used against it.

HISTORICAL.

De Senac (1749) (1) gave the first explanation of the pathogenesis of cyanosis. He considered it the result of an admixture of arterial and venous blood due to an abnormal communication between the two sides of the heart, which he found in autopsy. In 1761 Morgagni (2) showed that cyanosis might be combined with pulmonary stenosis. He explained the cyanosis by stasis caused by the pulmonary stenosis. Grancher (3) found a distention of the capillaries and the smallest arteries in patients suffering from (congenital) cyanosis, and considered this distention as well as the cyanosis as caused by stasis. Later Knapp (4) detected the alterations of the retinal vessels in patients with congenital cyanosis. This, together with experiments on animals by Panum (5), Dareste (6),

Stockard (7), Bardeen (8), Loeb (9), and others, resulted in giving the capillary anatomical changes more prominent and causal relation to cyanosis, which in some instances was considered due to anatomical malformation of the small vessels. Abnormalities in the blood itself have also been held responsible for the production of cyanosis. Since Krehl (10) found polycythemia in patients with congenital cyanosis and Vaquez (11) (1892) described the first case of polycythemia, an abnormally high number of red blood corpuscles has been considered a cause of cyanosis.

Alterations in the blood gases have also been considered the cause of cyanosis, and in spite of lack of experimental confirmation, this idea has never been given up. Some had the opinion that an accumulation of carbon dioxide in the blood, others that too small an amount of oxygen caused the cyanotic skin color. Also the amount of reduced hemoglobin has been considered the cause. Von Bamberger (12) early put this idea forward in his monograph on heart diseases. Since then, several others have used the same explanation, and it is usually considered one of the most important causes of cyanosis. No experimental proof has, however, been established. In a previous paper by the writer (13) it was pointed out that the results of a series of determinations of venous oxygen on decompensated heart patients seemed to show that there existed a close relation between cyanosis and the amount of reduced hemoglobin (called oxygen unsaturation) of the venous blood. Later Langstroth (14) confirmed this in two heart patients suffering from cyanosis. But the problem has never been submitted to a systematic investigation.

Present State of the Problem of the Cause of Cyanosis.

The literature shows that some confusion prevails, primary and secondary causes being intermixed. By primary causes I mean changes of the blood itself; by secondary causes I mean pathological disturbances, which may be more or less localized. Such intermixture of primary and secondary causes results in confusion such as we would have if we defined anemia as a condition caused by different factors; for instance, decreased hemoglobin, bleeding gastric ulcer, toxins, and so forth.

Primary Causes of Cyanosis.—Of what we may term primary causes, three are held especially responsible: (1) increased carbon dioxide content of the venous blood; (2) decreased oxygen content of the venous blood; and (3) increased oxygen unsaturation of the venous blood.[1]

[1] The term oxygen unsaturation has previously (15) been defined as "the difference between the venous oxygen and the total oxygen-combining power of the hemoglobin."

The chief aim of the present work has been to investigate whether or not any of these alterations of the blood really accounts for the cyanosis; *i.e.*, is the primary cause. For that purpose the cyanosis and the blood findings on a series of cyanotic and non-cyanotic individuals have been compared.

Technique.

The cyanosis is simply estimated (always in full daylight) and its degree indicated by plus signs. Three plus signs mean a very dark blue color. It has always been general (skin and mucous membranes) but never quite equally distributed. One plus indicates a just visible bluish color, usually but not always localized to the peripheral parts (acrocyanosis). Two pluses indicate an intermediate stage, in which the cyanosis is usually general but is pronounced only at certain places, such as the ears, lips, and finger-tips.

The blood samples have all been drawn and kept after the procedure given in previous publications (15, 16).

The total oxygen capacity is determined either directly by the Van Slyke method (17) or (in a minority of the cases) indirectly by calculation from the color index (Haldane's method). The venous oxygen and carbon dioxide are measured by the Van Slyke method (18). The carbon dioxide is determined on whole blood kept under oil for a very short time (19).

Determinations.

The results of the estimation of the cyanosis and the determination on the blood are given in Tables I to III. The figures of the first column indicate the serial number of the determination. In the second column are given in volumes per cent the values of the carbon dioxide (in Table I), the oxygen (Table II), and the oxygen unsaturation (Table III) of the venous blood. In Column 3 are the remarks on cyanosis, which are to be compared with the blood findings in Column 2. The figures in the fourth column indicate the serial numbers of corresponding determinations in Tables I to VI of Paper II (20), in which details about the patients and the blood determinations are given. In the last column are remarks about the condition of the individual from whom the blood has been drawn.

TABLE I.

Carbon Dioxide Content of the Venous Blood (Whole Blood Kept under Oil) in a Series of Cyanotic and Non-Cyanotic Individuals.

Determination No.	Carbon dioxide of venous blood kept under oil.	Cyanosis.	Serial No. of determination in Tables I to VI (Paper II).	Remarks (diagnosis, etc.).
	vol. per cent			
1	69.8	−	Not published.	Anemia (hemoglobin 44 per cent by Haldane's method).
2	67.7	−	47	Pneumonia.
3	62.1	+	4	Normal individual after exercise.
4	61.7	−	3	" " resting.
5	61.5	+++	48	Pneumonia.
6	58.2	+++	31	Normal individual; artificial stasis on arm.
7	58.1	−	9	Slightly decompensated heart failure; rest.
8	58.1	++	10	" " " " exercise.
9	57.0	−	Not published.	Normal individual; rest.
10	56.6	+	23	Decompensated heart failure.
11	56.6	+	30	Normal individual; artificial stasis on arm.
12	56.5	−	11	Decompensated heart failure; rest.
13	55.9	+	27	" " " "
14	55.6	++	12	" " " exercise.
15	55.4	+	24	" " " rest.
16	55.1	−	Not published.	Normal individual; rest.
17	54.7	++	28	Decompensated heart failure; rest.
18	54.1	−	Not published.	Normal individual; rest.
19	53.8	+	30	Decompensated heart failure; rest.
20	53.0	+	51	Normal individual inhaling air with low oxygen percentage.
21	52.1	−	50	Normal individual; rest.
22	51.9	−	29	" " "
23	51.5	+	34	Congenital heart failure; cyanosis.
24	51.3	+++	52	Normal individual inhaling air with low oxygen percentage.
25	50.8	−	49	Pneumonia.
26	50.5	−	1	Normal individual; rest.
27	50.5	+	2	" " exercise.
28	49.4	−	Not published.	Cardiac neurosis; tachycardia.
29	48.3	−	26	Compensated heart failure.
30	46.8	+++	38	Congenital " " cyanosis.
31	45.2	++	13	" " " "
32	44.6	−	Not published.	Normal individual; exercise.
33	44.1	+++	14	Congenital heart failure; cyanosis; exercise.
34	42.1	+++	39	" " " " "
35	41.5	++	35	" " " " rest.

TABLE II.

Oxygen Content of the Venous Blood in a Series of Cyanotic and Non-Cyanotic Individuals.

Determination No.	Oxygen content of venous blood.	Cyanosis.	No. of determinations in Tables I to VI (Paper II).	Remarks (diagnosis, etc.).
	vol. per cent			
1	0.00	++	12	Decompensated heart failure; exercise.
2	1.16	−	Not published.	Anemia (34 per cent hemoglobin by Haldane's method).
3	1.19	−	" "	Anemia (32 per cent hemoglobin by Haldane's method).
4	1.48	−	" "	Anemia (44 per cent hemoglobin by Haldane's method).
5	1.98	−	" "	Anemia (44 per cent hemoglobin by Haldane's method).
6	2.20	−	" "	Anemia (43 per cent hemoglobin by Haldane's method).
7	2.58	+	10	Decompensated heart failure.
8	3.14	+	20	" " "
9	3.79	+	15	" " "
10	3.95	−	Not published.	Anemia (60 per cent hemoglobin by Haldane's method).
11	4.10	+	22	Decompensated heart failure.
12	4.13	+	24	" " "
13	4.20	+	4	Normal individual; exercise.
14	4.29	+	19	Decompensated heart failure.
15	4.87	−	Not published.	Anemia (55 per cent hemoglobin by Haldane's method).
16	4.95	+++	45	Pneumonia.
17	5.09	−	Not published.	Anemia (55 per cent hemoglobin by Haldane's method).
18	5.23	+	17	Decompensated heart failure.
19	5.42	+	6	Normal individual; exercise.
20	5.72	+	2	" " "
21	6.32	?	21	Decompensated heart failure.
22	6.73	−	47	Pneumonia.
23	6.75	+++	43	"
24	7.00	+	27	Decompensated heart failure.
25	7.32	−	Not published.	Anemia (68 per cent hemoglobin by Haldane's method).
26	7.37	+++	52	Normal individual inhaling air with low oxygen percentage.
27	7.62	+	25	Decompensated heart failure.
28	7.76	+++	46	Pneumonia.

TABLE II—*Concluded.*

Determination No.	Oxygen content of venous blood.	Cyanosis.	No. of determinations in Tables I to VI (Paper II).	Remarks (diagnosis, etc.).
	vol. per cent			
29	8.05	−	11	Decompensated heart failure.
30	8.17	+++	31	Normal individual; artificial stasis on arm.
31	8.25	+	23	Decompensated heart failure.
32	8.52	+++	40	Pneumonia.
33	8.54	−	Not published.	Normal individual; slight exercise.
34	8.55	+	16	Decompensated heart failure.
35	8.62	+	28	" " "
36	8.78	?	8	Compensated " " exercise.
37	8.88	−	9	Decompensated " " rest.
38	9.09	+	30	Normal individual; artificial stasis on arm.
39	9.66	?	26	Decompensated heart failure.
40	9.80	+++	14	Congenital " " exercise.
41	10.13	−	Not published.	Anemia (93 per cent hemoglobin by Haldane's method).
42	11.40	++	34	Congenital heart failure.
43	11.40	+++	41	Pneumonia.
44	11.51	+++	39	Congenital heart failure.
45	12.26	−	49	Pneumonia.
46	12.62	+	51	Normal individual inhaling air with low oxygen percentage.
47	13.41	−	7	Compensated heart failure.
48	13.87	+++	38	Congenital " "
49	14.00	−	5	Normal individual; rest.
50	14.19	−	3	" " "
51	15.28	−	29	" " "
52	15.40	+++	37	Congenital heart failure.
53	16.20	−	26	Compensated " "
54	16.22	+++	48	Pneumonia.
55	16.31	−	1	Normal individual; rest.
56	17.03	−	50	" " "
57	17.44	+++	36	Congenital heart failure.
58	17.45	++	35	" " "
59	17.88	++	13	" " "
60	18.08	++	33	" " "
61	19.20	++	32	" " "
62	28.00	−	Not published.	Polycythemia (Vaquez' disease).

TABLE III.

Oxygen Unsaturation of the Venous Blood in a Series of Cyanotic and Non-Cyanotic Individuals.

Determination No.	Oxygen unsaturation of venous blood	Cyanosis.	No. of determinations in Tables I to VI (Paper II).	Remarks (diagnosis, etc.).
	vol per cent			
1	20.89	+++	39	Congenital heart failure.
2	19.82	++	12	Decompensated " " exercise.
3	18.48	+++	14	Congenital " " "
4	17.45	+++	38	" " " rest.
5	17.30	+	24	Decompensated " " "
6	17.24	+	10	" " " exercise.
7	16.62	++	34	Congenital " "
8	15.88	+++	37	" " "
9	15.41	+	15	Decompensated " "
10	14.68	+	2	Normal individual; exercise.
11	14.60	+	4	" " "
12	14.26	+++	36	Congenital heart failure.
13	14.02	+	27	Decompensated " "
14	13.97	+	19	" " "
15	13.86	+	30	" " "
16	13.62	+	6	Normal individual; exercise.
17	13.48	+	20	Decompensated heart failure.
18	13.43	+++	52	Normal resting individual inspiring air with low percentage of oxygen.
19	12.88	−	Not published.	Normal individual; exercise.
20	12.62	++	34	Congenital heart failure.
21	12.61	+++	45	Pneumonia.
22	12.53	+++	31	Normal individual; artificial stasis on arm.
23	12.52	+	22	Decompensated heart failure.
24	12.33	+	19	" " "
25	12.16	+++	42	Pneumonia.
26	12.15	+	23	Decompensated heart failure.
27	11.58	++	28	" " "
28	10.94	−	9	" " "
29	10.92	++	33	Congenital heart failure.
30	10.83	++	35	
31	10.81	+++	43	Pneumonia.
32	10.68	−	Not published.	Cardiac neurosis; exercise.
33	10.65	+	16	Decompensated heart failure.
34	10.47	?	8	" " " exercise.
35	10.40	++	13	Congenital " "

TABLE III—*Concluded.*

Determination No.	Oxygen unsaturation of venous blood.	Cyanosis.	No. of determinations in Tables I to VI (Paper II).	Remarks (diagnosis, etc.).
	vol. per cent			
36	10.30	?	21	Decompensated heart failure.
37	10.21	+	30	Normal individual; artificial stasis on arm.
38	9.90	++	32	Congenital heart failure.
39	9.54	?	18	Decompensated " "
40	9.40	−	Not published.	Normal individual; exercise.
41	8.86	+++	46	Pneumonia.
42	8.78	+++	48	"
43	8.68	+++	40	"
44	8.58	−	Not published.	Normal individual; exercise.
45	8.18	+	51	" " inhaling air with low oxygen percentage.
46	8.03	++	44	Pneumonia.
47	7.98	−	Not published.	Cardiac neurosis; rest.
48	7.93	++	41	Pneumonia.
49	7.12	−	Not published.	Normal individual; rest.
50	6.19	−	47	Pneumonia.
51	5.84	−	7	Compensated heart failure; rest.
52	5.20	−	5	Normal individual; rest.
53	5.01	−	3	" " "
54	4.82	−	26	" " "
55	4.72	−	29	" " "
56	3.79	−	1	" " "
57	3.77	−	50	" " "
58	2.35	−	49	" " "

In Table I are given the values in volumes per cent of the carbon dioxide of the whole (unchanged) venous blood. In twenty-three instances cyanosis of different degrees was encountered; in twelve instances no cyanosis was found. The table is arranged according to decreasing values of carbon dioxide. It is easily seen that no relation exists between the carbon dioxide content of the (venous) blood and cyanosis. A cyanotic color may be associated with low as well as with high values of carbon dioxide.[2]

[2] The upper normal limit of the carbon dioxide of the venous blood (whole blood drawn and kept under oil) is approximately 65 volumes per cent, the lower limit about 40 volumes per cent. Determinations of the carbon dioxide of whole blood in a series of individuals will be published later.

In Table II are given the values of the oxygen of the venous blood in volumes per cent. The table is arranged according to increasing values of oxygen content. A glance at the table shows that the amount of oxygen left in the venous blood[3] has no simple relation to the production of cyanosis. One may find cyanotic patients with very low values for the venous oxygen and others with very high values. As in Table I, the non-cyanotic are distributed over the same range.

The table shows that one may find very high (28.00 volumes per cent), as well as extremely low values for the venous oxygen. In one case (Determination 1) the venous blood contained no oxygen. The importance of this fact is discussed in a previous paper (21).

Table III presents the values of the oxygen unsaturation[4] arranged according to decreasing values. There is no doubt that some intimate relation exists between the oxygen unsaturation and the cyanosis. The most striking feature is undoubtedly that cyanosis is not associated with values of the oxygen unsaturation below about 8 volumes per cent (7.93 volumes per cent for No. 48).[5] This is of considerable importance because the upper limit of oxygen unsaturation in resting normal individuals has previously (16) been found to be about 8 volumes per cent.

This does not at all mean that we find a cyanotic color in all instances in which the oxygen unsaturation is above that limit. It is seen that values of the oxygen unsaturation from 8 to about 13.0 volumes per cent (12.88 volumes per cent for No. 19) may be encountered in cyanotic as well as in non-cyanotic individuals. Values above 13.0 volumes per cent have in all instances been associated with cyanosis. The highest observed value for the oxygen unsaturation is about 21 volumes per cent (20.89).

[3] In a previous publication (21) this is termed rest oxygen or reserve oxygen of the blood, analogous to the reserve force of the heart muscles.

[4] That is, the difference between the total oxygen capacity of the blood and the venous oxygen.

[5] In several other unpublished determinations of oxygen unsaturation the same holds true.

SUMMARY.

These data prove that abnormally high oxygen unsaturation of the blood is a cause of cyanosis.

The fact that the lowest value of oxygen unsaturation (in the venous blood) associated with cyanosis is about 8 volumes per cent seems to indicate that this amount of reduced hemoglobin is the lowest capable of producing a cyanotic color. We shall later discuss this point more in detail.

Table III shows furthermore that in spite of the fact that cyanosis is due to abnormally high oxygen unsaturation, no proportionality exists between the intensity of the blue color and the amount of reduced hemoglobin. This may in small part be due to individual peculiarities of the skin and subcutaneous tissue, which are known to influence in anemia the relation between paleness of the skin and the decrease in hemoglobin.

We shall, however, in the succeeding paper show that the main cause of the disproportionality between cyanosis and venous oxygen unsaturation is found in another factor, the recognition of which throws a clearer light on the pathogenesis of cyanosis, and explains why we may find values of oxygen unsaturation as high as 13 volumes per cent in non-cyanotic individuals.

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STUDIES ON CYANOSIS.

II. Secondary Causes of Cyanosis.

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By secondary causes of cyanosis we mean the conditions, physiological or pathological, which can increase the oxygen unsaturation of the venous blood to a degree great enough to give cyanosis. They may be divided into two groups according to the manner in which the oxygen unsaturation of the venous blood is increased: (1) conditions which increase the reduction of the normally saturated arterial blood in the capillaries; (2) conditions which prevent complete oxidation of the venous blood in the lungs.

To decide which of the two factors is responsible in a given case may be somewhat difficult. It could be done by drawing simultaneously samples of arterial and venous blood to find the degree of oxygen saturation in both. This I have not been able to do, because up to the present arterial punctures in patients have not been considered advisable.[1] We must in the present series of cases, therefore, confine ourselves to a judgment of the circumstances under which the cyanosis occurred. Sometimes in a given case it is difficult to decide exactly in which of the two ways cyanosis is produced. In other instances where the circumstances are less complicated, it can be done with certainty. The following is a report of a series of cyanotic patients grouped according to the way in which the cyanosis was produced.

The technique is described in the preceding paper.

[1] This is discussed in a previous publication (1) in which the determinations of the oxygen in the arterial blood by Hürter (Hürter, *Deutsch. Arch. klin. Med.*, 1912, cviii, 1) are quoted. In a recently completed work Dr. W. C. Stadie of the Hospital of The Rockefeller Institute has shown that arterial puncture can be safely performed (Stadie, W. C., *J. Exp. Med.*, 1919, xxx, 215).

Cases in Which the Abnormally High Venous Oxygen Unsaturation Is Probably Due to an Abnormally Great Deoxidation of the Blood in Passing through the Capillaries.

In normal resting individuals the reduction (deoxidation) of the arterial blood, which is supposed to leave the lungs nearly saturated with oxygen, may vary from about 8 to about 2.5 volumes per cent of oxygen, the average being 5.5 volumes per cent (1). The reduction is increased by exercise (2) if the increased oxygen consumption is not compensated by a faster blood flow through the capillaries. It is also increased in some heart diseases on account of slow circulation through the capillaries (1, 3, 4, 5). In some instances the reduction may be increased to such an extent that cyanosis occurs. Cases of both kinds (exercise and heart disease) are reported in Tables I to III. Nos. 1, 3, and 5 are determinations on normal resting individuals. The oxygen unsaturation and the carbon dioxide[2] of the venous blood are normal. Nos. 2, 4, and 6 are similar determinations on the same individuals immediately after exercise. The carbon dioxide is unchanged. The oxygen of the venous blood (reserve oxygen) has fallen to a rather low value, and consequently the oxygen unsaturation has increased considerably (to about 14 volumes per cent). In all instances a just visible cyanosis was discovered at the finger-tips immediately after exercise.

The fourth individual (Determinations 7 and 8) was a patient with a valvular disease compensated at rest. After slight exercise the oxygen unsaturation increased from the normal value to 10.47 volumes per cent. It was impossible to decide whether or not any cyanosis was produced by exercise in this case. Determinations 9 and 11 were done on a resting patient with a slightly decompensated valvular heart disease. The oxygen unsaturation is in both instances a little above the upper normal limit (8 volumes per cent). No cyanosis is present. After heavy exercise (Determinations 10 and 12) a moderate cyanosis was produced. The oxygen unsaturation showed a considerable increase (to about 17 and 20 volumes per cent). The carbon dioxide of the venous blood was a trifle lower after exercise.

[2] Carbon dioxide determination not done on the third patient.

The last individual (Determinations 13 and 14) was suffering from a congenital defect of the interventricular septum and congenital cyanosis. Even at rest he was markedly cyanotic with an oxygen unsaturation of only 10.40 volumes per cent. After heavy exercise the cyanosis had grown very intense and the oxygen unsaturation had increased to 18 volumes per cent. The carbon dioxide, which is low, was slightly decreased after exercise.

There is no doubt that the high oxygen unsaturation in the venous blood of the first three (normal) individuals after exercise (Table I) was due to increased deoxidation in the capillaries, for the reason that the lungs were and had always been perfectly normal. There appears to be no reason why the arterial blood should not be fully saturated. The same is probably the case with the next two patients, whose lungs also were normal at the period of determination. In the last case (Determinations 13 and 14) the matter is more complicated. On account of the communication between the two ventricles of the heart some blood must have been passing over into the arterial system without going through the lungs. This amount may possibly be increased by exercise and take a certain part in raising the unsaturation of the venous blood from 10 volumes per cent to 18 volumes per cent. The greatest part of the increase is, however, probably due to the increased reduction of the capillary blood by exercise. As to the way the deoxidation in the capillaries is increased, there are two possibilities; increased consumption on account of the greater metabolism, or increased deoxidation on account of slower blood flow. The blood samples were drawn from the arm after stair running; it seems unlikely that this form of exercise should be able to increase the metabolism in the arms, which were quiet. It is possible that a reflectory vasoconstriction of the capillaries in the unused areas —the arms—had produced a slower flow here in order to get more blood through the capillaries of the legs. Further investigations on this problem are in progress.

The main point is that it is experimentally shown that cyanosis can be produced by increased deoxidation in the capillaries, and that when due to this cause it seems to begin when the venous oxygen unsaturation approaches a value of 13 to 14 volumes per cent.

TABLE I.

Oxygen (Oxygen Unsaturation) and Carbon Dioxide of the Venous Blood in Normal Individuals and Patients with Compensated and Decompensated Heart Diseases at Rest and after Exercise.

Determination No.	Date.	Condition.	Total oxygen capacity (a).	Hemoglobin (calculated).	Oxygen content of venous blood (v).	Oxygen unsaturation (a-v).	Carbon dioxide of venous blood.	Pulse.	Respirations per min.	Remarks on cyanosis.	Conditions at the time of bleeding.	Diagnosis and clinical notes.
	1919		*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*					
1	Mar. 22	Rest.	20.10	108.5	16.31	3.79	50.5	90	18	No cyanosis.	Blood drawn after 10 min. rest in lying position.	
2		Exercise.	20.40	110	5.72	14.68	50.5	120		Slight cyanosis of fingers.	Blood drawn immediately after running up and down one flight five times in 90 seconds.	Normal individual, age 25 yrs.
3	Apr. 18	Rest.	19.20	104	14.19	5.01	61.7	80	14	No cyanosis.	The same as No. 1.	
4		Exercise.	18.80	102	4.20	14.60	62.1	120	24	Slight cyanosis of fingers.	" " " " 2.	Normal individual.
5	" 1	Rest.	19.20	104	14.00	5.20		62	15	No cyanosis.	" " " " 1.	
6		Exercise.	19.04	103	5.42	13.62		115	24	Slight cyanosis of fingers.	" " " " 2.	Normal individual, age 26 yrs.

7	Apr. 4	Rest.	19.25	104	13.41	5.84		84	20	No cyanosis.	The same as No. 1.	Male, age 26 yrs. Compensated mitral and aortic insufficiency; auricular fibrillation.
8		Exercise.	19.25	104	8.78	10.47		134	?	Doubtful cyanosis of fingers.	Blood drawn after walking up and down one flight twice.	
9	Feb. 20	Rest.	19.82	107	8.88	10.94	58.1			No cyanosis of hands or face.	The same as No. 1.	
10		Exercise.	(19.82)	(107)	2.58	17.24	58.1			Marked cyanosis of hands and face.	Blood drawn after walking up and down one flight five times.	Letter carrier, male, age 56 yrs. Compensated mitral insufficiency and stenosis.
11	" 21	Rest.	19.82	107	8.05	11.77	56.5			No cyanosis of hands or face.	The same as No. 1.	
12		Exercise.	19.82	107	0.00	19.82	55.6			Marked cyanosis of hands and face.	" " " " 10.	
13	Jan. 17	Rest.	28.28	153	17.88	10.40	45.2	82	18	Marked cyanosis of face and hands.	" " " " 1.	Male, painter, age 20 yrs. Congenital cyanosis. Defect of interventricular septum.
14		Exercise.	(28.28)	(153)	9.80	18.48	44.1	108	40	Heavy cyanosis of face and hands.	" " " " 10.	

TABLE II.

Oxygen and Carbon Dioxide of the Venous Blood in a Series of Patients with Decompensated Heart Diseases (All at Rest).

Determination No.	Date.	Total oxygen capacity (a).	Hemoglobin.	Oxygen content of venous blood (v).	Oxygen unsaturation (a–v).	Carbon dioxide of venous blood.	Pulse.	Respirations per min.	Remarks on cyanosis.	Diagnosis and clinical notes.
	1918	vol. per cent	per cent	vol. per cent	vol. per cent	vol. per cent				
15	Apr. 5	(19.20)	104	3.79	15.41		72*/66	34	Moderate cyanosis of hands and face.	
16	" 14	(19.20)	104	8.55	10.65		76/74	24	Moderate cyanosis of hands and face.	Housewife, age 64 yrs. Mitral insufficiency and stenosis; auricular fibrillation; no râles in lungs.
17	" 19	19.20	(104)	5.23	13.97				Moderate cyanosis of hands and face.	
18	" 26	(19.20)	(104)	9.66	9.54				Cyanosis doubtful.	
19	" 26	(16.62)	90	4.29	12.33		72/68	20	Slight cyanosis of hands and lips.	
20	May 2	(16.62)	(90)	3.14	13.48		144/108	22	Cyanosis more marked but still slight.	Housewife, age 42 yrs. Mitral insufficiency; aortic insufficiency; auricular fibrillation; no râles in lungs.
21	" 5	(16.62)	(90)	6.32	10.30		92/90	22	Cyanosis doubtful.	
22	" 10	(16.62)	(90)	4.10	12.52		72/72	22	Slight cyanosis.	

23	1919 Jan. 25	20.40	(110)	8.25	12.15	56.6	132/96	28	Moderate cyanosis of face and hands.	
24	" 27	21.43	(116)	4.13	17.30	55.4	128/80	30	Moderate cyanosis of face and hands.	
25	" 30	21.48	(116)	7.62	13.86	53.8	102/90	32	Moderate cyanosis of face and hands.	Housewife, age 44 yrs. Mitral insufficiency and stenosis; auricular fibrillation; no râles in lungs.
26	Feb. 1	21.02	114	16.20	4.82	48.3	100/94		No cyanosis.	
27	" 4	(21.02)	(114)	7.00	14.02	55.9	102/92	24	Moderate cyanosis of face and hands.	
28	Apr. 15	20.20	(109)	8.62	11.58	54.7	—/40	22	Marked cyanosis of face and hands.	Mitral stenosis; auricular fibrillation; no râles in lungs.

* The upper figure is the apex pulse, the lower the radial pulse.

In Table II a report is made of fourteen determinations on four patients with valvular heart lesions combined with auricular fibrillation. In one of these determinations an oxygen unsaturation of normal value (No. 26) was found. The patient showed no cyanosis and was fully compensated at that time. In all the other instances the oxygen unsaturation was above the upper normal limit and the patients were clinically decompensated. In eleven instances a cyanosis of slight or moderate degree was encountered In two instances (Nos. 18 and 21) it could not be determined whether or not there was cyanosis. In these two instances the oxygen unsaturation was 9.54 and 10.30 volumes per cent. During the cyanotic periods in the case covered by Determinations 15 to 18 the oxygen unsaturation was from 10.65 to 15.41 volumes per cent.

Although it seems to be almost certain that the circulation in the instances of cyanosis shown in Table II was slower than normally and that the deoxidation consequently is due to slower blood flow and not to increased metabolism, it cannot be excluded that a part of the oxygen unsaturation of the venous blood may have been due to incomplete oxidation in the lungs. In spite of the fact that the lungs in all instances were perfectly clear, it might be possible that their function had been impaired from earlier periods of stasis.

It is probably justifiable to say that the cyanosis in these cases was produced mainly, although not exclusively, by increased deoxidation on account of slow circulation. The lowest value of oxygen unsaturation at which cyanosis was found was 10 to 11 volumes per cent, somewhat lower than in the experiments with exercise, a point which will be discussed later.

In Table III three experiments on a normal person (the writer) are reported. No. 29 is a determination of the oxygen and carbon dioxide of the venous blood at rest. The values obtained are normal. Then the blood flow was stopped in the arm by stasis by means of the blood pressure apparatus. For 2 minutes the pressure was kept at 30 mm. of mercury, allowing the arterial stream to get through; then the pressure was raised to 160 mm. (the blood pressure of the individual was $\frac{125}{80}$) for 2 minutes, and a blood sample was drawn. The analysis showed that the oxygen unsaturation had increased from 4.7

TABLE III.

Oxygen and Carbon Dioxide of the Venous Blood after Stopping of the Blood Flow in the Arm of a Normal Individual (the Writer).

Determination No.	Date.	Time.	Total oxygen capacity (a).	Hemoglobin (calculated).	Oxygen content of venous blood (v).	Oxygen unsaturation (a-v).	Carbon dioxide of venous blood.	Remarks on cyanosis of arm.	Pressure in apparatus when blood sample was drawn.	Ordinary blood pressure of the subject.
	1919	*p. m.*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		*mm.*	*mm.*
29	Mar. 13	3.10	(20.00)	(108)	15.28	4.72	51.9	No cyanosis.	0	$\frac{125}{80}$
30		3.14	19.30	104	9.09	10.21	56.6	Slight cyanosis; veins swollen.	160	
31		3.18	20.70	112	8.17	12.53	58.2	Marked " " "	160	

volumes per cent to 10 volumes per cent, and a slight cyanosis was present in the whole arm. After stasis for 4 minutes more the cyanosis was very marked; the oxygen unsaturation had risen to 12.5 volumes per cent. The stasis had increased the carbon dioxide content from 51.9 volumes per cent to 58.2, both of which values lie within the normal limits.

This experiment shows the production of cyanosis by increased deoxidation of the arterial blood in the capillaries. It is striking that the cyanosis starts at a lower unsaturation (10.2 volumes per cent) than did the cyanosis in the experiments with exercise (13 to 14 volumes per cent). It is especially noteworthy that the cyanosis when caused by retarded circulation became intense at an oxygen unsaturation of 12.5 volumes per cent, which is not much higher than the value at which it was just visible when caused by exercise. This point is discussed later.

Cases in Which Cyanosis Is Exclusively or Chiefly Due to Incomplete Oxidation of the Blood in the Lungs.

Ordinarily the arterial blood is approximately saturated with oxygen. In some instances, however, the anatomical or physical conditions are changed in such a way that complete saturation does not take place. This is the case (1) when a part of the blood does not pass the lungs on account of an abnormal communication between the arterial and venous part of the vascular system, for instance a patent ductus Botalli, or a deficient septum of the ventricles of the heart; (2) when a part of the lungs is unfitted for the aeration but still allows the blood flow, for instance in pneumonia; (3) when the partial oxygen pressure in the lungs is below a certain point, for instance at high altitudes, mountain sickness; and (4) when a part of the hemoglobin is transformed into such a form that it cannot be converted into oxyhemoglobin and still gives a dark color like reduced hemoglobin, as in methemoglobinemia.[3] In all these instances (the first three of

[3] In methemoglobinemia, which may give an intense cyanosis, it is not quite correct to define the cyanosis as due to increased oxygen unsaturation, for the reason that a certain part of the hemoglobin can neither take up nor give off oxygen.

which are represented by experiments in this publication) the oxygen saturation of the arterial blood is incomplete and there is a non-negligible oxygen unsaturation of the arterial blood. In the capillaries this (arterial) oxygen unsaturation is further increased on account of the (normal or abnormal) deoxidation. The oxygen unsaturation in such cases is therefore a result of two factors, (1) incomplete oxidation in the lungs and (2) reduction in the capillaries.

In a series of twelve patients and one normal individual the clinical and experimental conditions have been such that although we were unable to determine the degree of arterial unsaturation we are certain that arterial unsaturation is the cause of the abnormally high venous oxygen unsaturation causing the cyanosis. The cases fall into three groups and are reported accordingly.

In Table IV a report is given of nine determinations of the blood gases in two patients, both suffering from congenital heart disease (defective ventricular septum). In both cases rather high values for the oxygen of the venous blood were found, from 11 to 19 volumes per cent, in spite of the fact that the oxygen unsaturation of the venous blood showed values which in some instances were higher than those seen in any other case, from 9.9 to 20.89 volumes per cent. The carbon dioxide of the venous blood showed values at the very lowest limit of what can be considered normal.

The cyanosis in the first case in Table IV was fairly moderate, in the last case extremely heavy. No doubt existed that the unusually high oxygen unsaturation was not due to increased deoxidation in the capillaries, because the determinations were done after half an hour's rest, and no clinical reason for slow circulation was to be detected (no stasis, no edema, diuresis normal). The cause of increased venous oxygen unsaturation must therefore lie in incomplete oxidation in the lungs[4] caused either by some lung disease or by a congenital heart lesion. The lungs were absolutely normal, and clinically a defect of the interventricular septum was found.

In Table V a report is given of ten determinations on ten patients suffering from pneumonia (post influenza). In one case (No. 49) the oxygen unsaturation of the venous blood was very low, 2.35 volumes per cent, which is a trifle below the lower normal limit (2.5 volumes

[4] No reason existed for considering methemoglobinemia the cause.

TABLE IV.

Oxygen and Carbon Dioxide of the Venous Blood of Two Patients Suffering from Defect of the Interventricular Septum, Congenital Cyanosis, and Polyglobulism.

Determination No.	Date.	Total oxygen capacity (a).	Hemoglobin.	Oxygen content of venous blood (v).	Oxygen unsaturation (a-v).	Carbon dioxide of venous blood.	Pulse.	Respirations per min.	Remarks on cyanosis.	Diagnosis and clinical notes.
	1918	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*				
32	Oct. 22	(29.10)	156	19.20	9.90		86	20	Moderate cyanosis, especially of face, hands, and feet.	Male, painter, age 20 yrs. Congenital heart disease (defect of the interventricular septum); congenital cyanosis; pulmonary stenosis (?). Number of red corpuscles varied between 6.2 and 7.2 millions. No stasis or edema. Superficial veins a little more marked than usual. Veins of retina dilated. Slight clubbing of fingers and toes.
33	Nov. 5	29.00	(156)	18.08	10.92		86	20		
34	" 16	(28.02)	151	11.40	16.62	51.5	84	24		
	1919									
35	Jan. 16	28.28	(153)	17.45	10.83	41.5	84	22		
13	" 17	(28.28)	(153)	17.88	10.40	45.2	82	18		
	1918									
36	Sept. 19	31.70	(170)	17.44	14.26		100	24	Extremely heavy cyanosis of the skin of the whole body and of the mucous membranes.	Male, age 7 yrs. Congenital heart disease (defect of the interventricular septum); congenital cyanosis. Number of red blood corpuscles varied from 7 to 10 millions. No stasis or edema. Heavy dyspnea, increasing markedly under exercise. Superficial veins marked. Veins of retina dilated. Marked clubbing of fingers and toes.
37	" 23	(31.08)	168	15.40	15.68		90	26		
38	Dec. 15	31.32	(169)	13.87	17.45	46.8	108	36		
	1919									
39	Feb. 2	32.40	(175)	11.51	20.89	42.1	86	34		

TABLE V.

Oxygen and Carbon Dioxide of the Venous Blood in a Series of Patients Suffering from Pneumonia (in Influenza).

Determination No.	Date.	Total oxygen capacity (a).	Hemoglobin.	Oxygen content of venous blood (v).	Oxygen unsaturation (a-v).	Carbon dioxide of venous blood.	Pulse.	Respirations per min.	Temperature.	Degree of cyanosis.	Diagnosis and clinical notes.
	1918	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*			*°C.*		
40	Oct. 15	(17.20)	93	8.52	8.68		92	38		+++	Male, student, age 16 yrs. Double pneumonia.
41	" 22	(19.33)	104	11.40	7.93		96	50		++	Female, age 38 yrs. Double pneumonia.
42	" 23	(15.72)	85	3.56	12.16		132	48	40.8	+++	" " 23 " " "
43	" 23	(17.56)	95	6.75	10.81		120	36	40.6	+++	" " 23 " " "
44	" 29	(14.79)	80	6.76	8.03		86	48	39.7	++	" " 39 " " " mitral and aortic stenosis.
45	" 30	(17.56)	95	4.95	12.61		120	40		+++	Female.
46	" 31	(16.62)	90	7.76	8.86		100	50	40.3	+++	" age 29 yrs. Double pneumonia.
47	Nov. 9	(12.92)	70	6.73	6.19	67.7	124	36	40.0	–	Age 16 yrs. Double pneumonia.
48	Dec. 1	25.00*	(135)	16.22	8.78	61.5	84	24	39.0	+++	Male, porter, age 43 yrs. Double pneumonia.
49	" 4	(14.61)	80†	12.26	2.35	50.8	84	28	38.8	–	Female, housemaid, age 19 yrs. Pneumonia involving left lower lobe.

* Total oxygen capacity directly determined (Van Slyke's method).

† Total oxygen capacity calculated from hemoglobin determination (Sahli).

In the rest the total oxygen capacity was calculated from the hemoglobin percentage determined by Haldane's method. Nos. 41 and 49 recovered; the rest died from pneumonia.

per cent). This patient was not cyanotic. In another (No. 47) the unsaturation value was within normal limits, and just above the normal average. No cyanosis was present in this patient either.

The other eight patients were cyanotic, most of them markedly so. In these cases the oxygen unsaturation of the venous blood had values from 7.93, a trifle below the upper normal limit, to 12.61 volumes per cent. As a whole, the oxygen unsaturation is abnormally high in the venous blood from the cyanotic individuals, but by far not to the same degree as was found in normal individuals after exercise, although the cyanosis in pneumonia was much more intense and generalized. In three patients, two non-cyanotic and one cyanotic, the carbon dioxide was determined. In one of these (No. 47, non-cyanotic), it was just above the upper normal limit. In the other two it was normal. The total oxygen-combining power of the blood was in one case (No. 48) very high, corresponding to a hemoglobin percentage of 135 (verified twice). In another case (No. 41) it was a little above normal. In the rest of the cases it was decreased (hemoglobin from 95 to 70 per cent).

The decision of the pathogenesis of cyanosis in these cases is somewhat difficult for several reasons. In the first place, it is possible that it might in part be due to formation of methemoglobin. From the investigations of Butterfield and Peabody (6) it is known that a discrepancy between the amount of iron in blood and its oxygen-combining power may be produced by pneumococci on account of formation of methemoglobin. No determinations of the bacteria in the blood of the present pneumonia patients were done.

If a part of the hemoglobin is transformed into methemoglobin it may or may not be included in the oxygen unsaturation values. That depends on the method used in determining the total oxygen-combining power. If this is done directly by the Van Slyke method (7) only that part of the hemoglobin which can take up and give off oxygen is included.

By the Haldane or Palmer method a mixture of carbon monoxide hemoglobin and methemoglobin is obtained which cannot be accurately matched against the standard of pure carbon monoxide hemoglobin. Neither is it possible by the Sahli method to determine accurately the total hemoglobin if a part of it is transformed into

methemoglobin.[5] In the determinations in Table V Haldane's method was used in eight cases, seven of which had cyanosis, Van Slyke's method in one (No. 48), and Sahli's in one (No. 49) in which no cyanosis was present. In one case (No. 41) blood and urine were examined spectroscopically. No methemoglobin was detected. This means that there is a possibility that methemoglobin may have been partly responsible for the cyanosis, although it cannot be seen from the oxygen saturation values. The rather low values for the total oxygen-combining power in some of these cases might also be interpreted as caused by transformation of some part of the hemoglobin into methemoglobin, although this is entirely conjecture.

However, the main part and probably the whole of the cyanosis must be due either to increased reduction or incomplete oxidation of the blood. As to the first possibility, nothing could be detected in the circulation signs which could indicate slow circulation (no stasis, pulse powerful, the mitral stenosis found in one patient was compensated), and the patients were all resting. But the temperature was increased, which undoubtedly caused an increase in the metabolism and this means again an increased oxygen consumption in the tissues. That fever does not necessarily cause an increased oxygen unsaturation of the venous blood is seen in No. 49, where it was even subnormal in spite of a temperature of 38.8°C. This probably means that the increased activity of the heart, which was clinically very marked, had overcompensated the increased oxygen consumption, so that the venous blood was even more arterialized than normally. It is probably justifiable to ascribe the cyanosis in these cases to incomplete oxidation of the venous blood in the lungs on account of the pneumonic infiltration, which allowed the blood to pass but did not permit the air to come in contact with it. As in the patients with congenital cyanosis, the blue color is more general and begins at a lower oxygen unsaturation value than is the case in individuals when cyanosis is caused by increased reduction of hemoglobin in the capillaries.

This is also seen in an experiment in Table VI in which the influence of low oxygen pressure on the oxygen unsaturation of the venous blood is investigated. The experiment was arranged as follows: The subject

[5] Stadie, W. C., personal communication.

TABLE VI.

Effect of Inspiration of Decreasing Amounts of Oxygen on the Skin Color and on the Oxygen and Carbon Dioxide of the Venous Blood of a Normal Individual (the Writer).

Determination No.	Date.	Time.	Total oxygen capacity (a).	Hemoglobin (calculated.)	Oxygen content of venous blood (v).	Oxygen unsaturation (a-v).	Carbon dioxide of venous blood.	Pulse.	Respirations per min.	Oxygen content of expiratory air.	Carbon dioxide content of expiratory air.	Remarks on cyanosis.
	1919	*p. m.*	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*			*per cent*	*per cent*	
50	Mar. 18	3.28	20.80	(112)	17.03	3.77	52.1	88	17			No cyanosis.
51		3.45	(20.80)	(112)	12.62	8.18	53.0	90	24	5.68	3.69	Cyanosis starting (hands, arms, and face).
52		3.48	(20.80)	(112)	7.37	13.43	51.3	90	28	5.53	2.77	Cyanosis of skin and mucous membranes very marked.

(the writer) was lying on a bed. Through a mask respiration took place from a spirometer with a capacity of about 30 liters. By means of a double valve arrangement the exposed air on its way to the spirometer passed a tower filled with soda lime, which absorbed the carbon dioxide. By thus rebreathing into the spirometer the oxygen percentage of the air decreased regularly and no increase in the carbon dioxide took place. A propeller mixed the air in the spirometer.

In several preliminary experiments the same results were obtained as in Haldane's (8). After a certain period of rebreathing the individual became cyanotic. An analysis of the air in the spirometer would then show between 8 and 5.5 per cent oxygen; the carbon dioxide about 0.5 volume per cent. The cyanosis usually started after rebreathing for about 20 minutes, and a few minutes later it would be impossible for the individual to continue on account of dizziness, throbbing in the head and extremities, palpitation, and a choking feeling.

In the experiment reported in Table VI a sample of blood was drawn from an arm vein before the spirometer breathing started. Then the respiration from the spirometer began without any change in the position of the individual on whom the experiment was performed. After 20 minutes a slight but distinct cyanosis was to be seen on the arms and cheeks. A sample of air from the mask just outside the mouth was drawn and at the same time a blood sample was taken.

The experiment was now pushed on as far as possible and three minutes after the drawing of the second sample of blood a third sample was taken together with an air sample. At that time the cyanosis was very marked and could be seen in the skin and the mucous membranes. Although it was generalized it was most conspicuous on the hands, cheeks, nose, and ears. At this time the individual was nearly fainting and felt very uneasy. The pulse rate had increased a little and the respiration had gone up from 17 to 28.

The analyses show that the cyanosis started at a venous oxygen unsaturation of 8.18 volumes per cent and was very heavy at 13.43 volumes per cent. The oxygen of the venous blood had decreased from 17.03 volumes per cent to 7.37 volumes per cent. The carbon dioxide was practically uninfluenced and showed a normal value.

The pathogenesis of the low venous oxygen unsaturation is clear. Nothing occurred which could give an increased deoxidation in the capillaries. But oxidation in the lungs must have been incomplete on account of the low oxygen percentage found in the lung air.

It is impossible to give the exact figure of the alveolar oxygen pressure because it was impossible to get a deep expiration on account of the dyspnea. The air sample, which was taken from just outside the mouth of the individual simultaneously with the drawing of the second and third blood samples, therefore shows oxygen values a little higher than the alveolar air. The oxygen percentage was, in the first sample, 5.68, in the second 5.53; the corresponding pressure was 39.7 and 38 mm. of mercury. From this we can calculate the approximate saturation of the arterial blood, using the normal average curve for the dissociation of the oxyhemoglobin. The degree of saturation could not be far from 70 per cent. Since the total oxygen-combining power is 20.80, this gives an oxygen content of the arterial blood of 15.25 volumes per cent. The difference is the oxygen unsaturation of the arterial blood, 5.55 volumes per cent. The oxygen unsaturation of the venous blood was 8.18 volumes per cent; of this 5.55 are consequently due to arterial unsaturation, whereas 2.63 volumes per cent are the result of the reduction in the capillaries on account of the oxygen consumption.

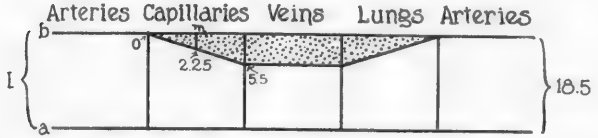
DISCUSSION.

It has been shown in this and the preceding paper (1) that an abnormally high oxygen unsaturation of the blood results in the clinical condition known as cyanosis;[6] (2) that this increased oxygen unsaturation may be produced in two ways, (*a*) by an increased reduction of oxyhemoglobin to reduced hemoglobin in the peripheral capillaries, and (*b*) by an incomplete oxidation of the venous blood in the lungs.

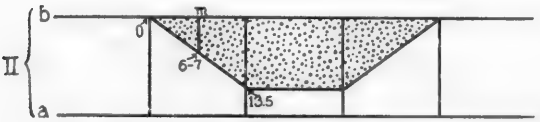
It was pointed out that no quantitative parallelism exists between the degree of cyanosis and the amount of oxygen unsaturation of the venous blood. This lack of parallelism was first thought to be

[6] The possible production of cyanosis by methemoglobinemia has been mentioned but not discussed in detail as no data are available.

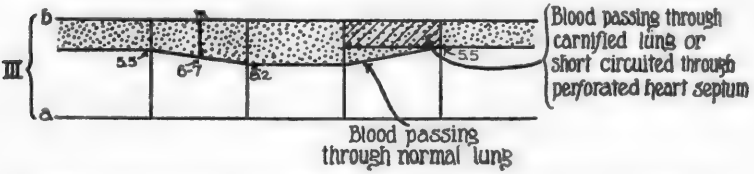
due to individual differences; for instance, of the skin and the subcutaneous tissue. However, the separation of the cyanotic patients into two groups according to the way the cyanosis was produced (the secondary causes) seemed to offer an opportunity for a better understanding of the problem. The results on the two groups of cyanotic patients seemed, as previously mentioned, to indicate that



Oxygen unsaturation for normal individuals.



Oxygen unsaturation when the reduction of hemoglobin in the peripheral capillaries is abnormally increased.



Oxygen unsaturation when the oxidation of hemoglobin in the lungs is incomplete.

TEXT-FIG. 1. Oxygen unsaturation of the blood in the different parts of the vascular system under various conditions. Total areas between lines *a* and *b* represent oxygen capacity, shaded areas oxygen unsaturation.

there was an important difference between them. In the first group the cyanosis was as a whole local and not very intense, beginning at a rather high degree of oxygen unsaturation of the venous blood, whereas in the second group it was usually generalized, often very intense, and it seemed to start at a low degree of oxygen unsaturation.

Text-fig. 1 represents the conditions in the different parts of the circulatory system. The distance from *a* to *b* represents the total

oxygen-combining power of the blood. The arterial system, the peripheral capillaries, the venous system, and the lungs are represented as indicated on the figure. Diagram I gives the condition in normal resting individuals. In the arteries the hemoglobin is approximately saturated with oxygen. The proportion of hemoglobin which is saturated with oxygen is represented by the white areas. In the capillaries a certain amount of oxygen is taken away by the tissues, and the highest degree of unsaturation is found at the end of the capillaries (on the average 5.5 volumes per cent). This unsaturation is unchanged until the venous blood reaches the lungs and is again oxidized.

Diagram II represents conditions in individuals, in whom the oxygen unsaturation of venous blood for some reason is increased on account of increased deoxidation in the capillaries (after exercise, slow circulation).

Diagram III gives a picture of the condition in which only part of the reduced hemoglobin of the venous blood is changed to oxyhemoglobin in the lungs. There is consequently a certain proportion of reduced hemoglobin in the arterial blood when it enters the tissue capillaries. This is further increased by the deoxidation normally occurring in the capillaries. The sum of arterial unsaturation plus reduction in the capillaries is the venous oxygen unsaturation. This condition may be found in pneumonia, some congenital heart diseases, and after respiration of air with low oxygen percentage.

All the blood samples were drawn from the veins, and the results of the analyses of these samples are compared with the cyanotic color. It is clear, however, that it is not the blood in the venous, or in the arterial system which causes the cyanosis; it is the color of the blood in the capillaries. But any part of the capillaries lying sufficiently superficially must take the same part in producing the skin color, which is to be looked upon as the result of a mixture of the condition in any small sector of the capillaries. In other words, it must be the sum of these sectors, which is responsible for the cyanosis. As to cyanosis, the sum is approximately represented by the average between the value at the beginning and the end of the capillaries. If we can calculate this average, indicated by the line *m* on the diagram, we may find the real oxygen unsaturation which is responsible

for the cyanosis. In normal individuals the values for the oxygen unsaturation at the beginning and end of passage through the capillaries are approximately 0 and 5.5 volumes per cent respectively. This indicates an average capillary oxygen unsaturation of 2.25 volumes per cent.

As an example of the condition represented in Diagram II, we shall take the results on the normal exercising individuals. The oxygen unsaturation of the arterial blood is about 0 volume per cent, that of the venous blood about 13 to 14 volumes per cent. This gives a mean capillary oxygen unsaturation of about 6.5 to 7 volumes per cent.

Diagram III may be applied to the experiment with the inspiration of air with low oxygen percentage. The arterial oxygen unsaturation was calculated to be not far from 5.55 volumes per cent. The venous oxygen unsaturation at the beginning of the cyanosis was 8.18 volumes per cent. The average is between 6 and 7 volumes per cent. In other words, the average capillary oxygen unsaturation that initiated barely visible cyanosis in selected examples from both groups, was between 6 and 7 volumes per cent, which consequently is to be looked upon as the threshold value for the cyanosis.

This view-point gives us means to a better judgment of the two factors which usually are alone available, the cyanosis and the venous oxygen unsaturation. If a cyanosis starts at a venous oxygen unsaturation lower than 13 volumes per cent, some arterial oxygen unsaturation must be present, and the more the lower the venous unsaturation is. This is often seen in heart patients, particularly in patients suffering from mitral disease, rarely in patients with aortic involvement. It is seen even if no râles are heard and no dullness is present. Almost all the results in Table II show this condition. This means that the incomplete oxidation causing arterial oxygen unsaturation plays a very important part and may be present even if no physical signs of lung involvement can be detected.

This also explains why the cyanosis in pneumonia patients starts at a very low degree of venous oxygen unsaturation and indicates that it is the lung ventilation and not the circulation which is impaired in these cases. In one patient (No. 41) the cyanosis was well developed at the venous oxygen unsaturation of 7.93 volumes per cent. This means that the arterial oxygen unsaturation was at

least between 5 and 6 volumes per cent, and from this it can be calculated that about one-third of the blood passed through lung tissue which was not ventilated.

In the experiments with artificial stasis of the arm the cyanosis begins at a venous oxygen unsaturation of 10.21 volumes per cent. This, on the basis of the above theory, would indicate the presence of some arterial unsaturation. However, it is unlikely that the arterial blood was not practically saturated with oxygen in the subject. The determination (No. 29) 4 minutes before showed that the arterial blood in this individual must have been saturated. The explanation is that back pressure from the veins caused stasis in the capillaries and reduction of an unusual proportion of the oxyhemoglobin on entering the first parts of the capillaries. Consequently the condition simulates that observed when incomplete oxygenation of the arterial blood is the cause of the presence of reduced hemoglobin at the entrance to the capillaries.

Still another phenomenon finds its explanation from the fact that it is the average capillary oxygen unsaturation which parallels the degree of cyanosis. It has been pointed out before that there must necessarily be a certain amount of hemoglobin in the blood to cause cyanosis; in other words, that patients suffering from anemia above a certain degree could not turn cyanotic. This limit can be determined by applying artificial stasis to such patients. In a series of such patients it was found that the threshold value was about 35 per cent hemoglobin, or a total oxygen-combining power of about 6.5 volumes per cent.

SUMMARY.

1. The primary cause of cyanosis is an increase in the reduced hemoglobin, or oxygen unsaturation, of the blood in the peripheral capillaries.

2. When the mean capillary oxygen unsaturation, which is calculated as the mean between venous and arterial unsaturation, and is normally about 2 to 3 volumes per cent, is increased to about 6 to 7 volumes per cent, cyanosis appears. For this reason 6 to 7 volumes per cent may be called the threshold value of mean capillary oxygen unsaturation for the incidence of cyanosis.

3. The increased mean capillary oxygen unsaturation is produced in two ways (secondary causes of cyanosis), either by an abnormally great reduction during passage through the capillaries (Text-fig. 1, Diagram II) or by a state of partial reduction in the arterial blood entering the capillaries (Text-fig. 1, Diagram III). The first condition (abnormally great reduction) occurs during exercise, or when the blood flow is retarded, as in decompensated heart condition. The second condition (partial arterial unsaturation) occurs in certain lung and heart diseases, and when the alveolar oxygen tension is greatly decreased, as at high altitudes.

4. If the blood is completely saturated with oxygen in the lungs, the oxygen unsaturation of the venous blood may increase to 13 to 14 volumes per cent before cyanosis appears.

5. If cyanosis appears at a venous oxygen unsaturation less than 13 to 14 volumes per cent, some arterial oxygen unsaturation may be assumed, and the more the lower the venous oxygen unsaturation is.

6. Even if neither râles nor dullness can be detected in the lungs, conditions may exist which prevent complete oxidation of the arterial hemoglobin. This is especially frequent in patients with mitral lesions.

7. Cyanosis cannot be produced in patients whose hemoglobin percentage is below 35 per cent on the Haldane scale (oxygen capacity of 6.5 volumes per cent).

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ERYTHROSIS, OR FALSE CYANOSIS.

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A general increase above the normal figure in the number of red blood corpuscles—polycythemia, erythremia—is met with under three conditions: (1) at high altitudes; (2) in certain heart diseases, particularly in congenital forms; (3) in polycythemia vera cum splenomegalia—Vaquez' disease.

Individuals with polycythemia of sufficient degree usually have an abnormal skin color. In some cases they have a reddish, in others more of a bluish color. There is in the literature some confusion as to the classification of this color. It is especially questionable whether the color in these patients is to be called cyanosis or not. The reason for the difficulty seems to be (1) the historic development of the nosology of the diseases in which polycythemia is found, and (2) the lack of a precise physiological explanation of cyanosis.

As to the first point a few historical remarks are necessary.

It is well known that a quick change from a low to a high altitude causes a series of symptoms which we call mountain sickness. The study of this disease was put on a scientific basis by Jourdanet (1) in 1864 and further developed by Paul Bert (2). The polycythemia was suggested by Paul Bert in 1882 and demonstrated by Viault (3) in 1891. Since then numerous investigators, especially Zuntz and Loewy (4) and Douglas, Haldane, Henderson, and Schneider (5) have studied this condition. Two of the main symptoms are polycythemia, and under certain circumstances blueness of the skin—cyanosis.

The polycythemia in heart diseases was first shown by Toenissen (6) in 1881 and further developed by Krehl (7), who in 1889 described a patient with polycythemia, cyanosis, and congenital heart disease with deficient septum ventriculorum. Several cases have since been described. The most frequent symptoms in congenital heart disease with deficient septum ventriculorum are polycythemia and almost constant cyanosis.

Vaquez (8) in 1892 described a patient with polycythemia who had an abnormal skin color, which he called cyanosis. He considered it a case of congenital heart failure. The postmortem examination (1893) showed no abnormalities in the heart, but an extensive enlargement of the spleen. Since then several similar cases have been reported, and this symptom complex is now recognized as a special clinical entity (Osler, 1903 (9)).

In the first description the skin color of erythrosis was called cyanosis. Chiefly for this reason, probably, most subsequent authors have described the color as cyanotic, although some of the later writers, for instance Osler and McCrae, have been inclined to describe the color not as a cyanosis, but as a congestion or ruddiness of the skin. Another reason for the confused nomenclature is probably, as already mentioned, the uncertainty in our understanding of the cause of cyanosis.

In accompanying papers (10) it is shown that the cause of cyanosis is the increase in the oxygen unsaturation of the venous blood, or rather of the blood in the capillaries. It is probably justifiable to put cyanosis in the same relation to oxygen unsaturation that anemia is to hemoglobin.

It is, therefore, of importance to determine the oxygen unsaturation in such cases. In this paper a report is made of the determination of the oxygen unsaturation in a typical case of Vaquez' disease.

The patient was a man aged 43 years. He was admitted to the hospital for duodenal ulcer. In the hospital a considerable enlargement of the spleen was found, with 181 per cent hemoglobin, 10,900,000 red blood corpuscles, 3,000 leucocytes, blood pressure 150 mm., Wassermann reaction negative. The skin and mucous membranes of the eyes, mouth, and rectum showed a deep red color.

Four determinations of the oxygen unsaturation and three of the carbon dioxide of the venous blood were done (Van Slyke's methods were used (11–13)). The results are given in Table I.

The values of the oxygen content in the venous blood are considerably greater than we usually find in normal individuals. They are increased in the same degree as the total oxygen-combining power of the blood. The oxygen unsaturation is consequently normal. The values for the carbon dioxide are low, one of them at the lowest normal limit. This may be due to the relative decrease in the serum of the

TABLE I.

Oxygen Unsaturation and Carbon Dioxide Content of the Venous Blood in a Patient with Vaquez' Disease.

Determination No.	Date.	Total oxygen capacity, directly determined (Van Slyke's method) (a).	Hemoglobin (calculated).	Oxygen content of venous blood (v).	Oxygen unsaturation (a–v).	Carbon dioxide content of whole blood.	Pulse.	Respirations per min.
	1919	*vol. per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*		
1	Jan. 8	31.50	170	26.20	5.30		70	15
2	" 13	31.50	170	26.50	5.00	58.0	66	14
3	" 21	33.40	181	28.00	5.40	56.6	68	16
4	Feb. 4	31.64	171	25.01	6.63	45.5	70	16

blood and to the abnormally high hemoglobin content of the venous blood. Neither in the skin color nor in the blood findings was any reason found for considering this man cyanotic. It was, however, evident that his color was not normal but reddish. When we consider that this particular color is one of the cardinal symptoms of the disease, it seems justifiable to give it a definite name. For this reason it is proposed to call the condition in which this color appears by the name "erythrosis." The three main symptoms of Vaquez' disease are then (1) polycythemia, (2) erythrosis of the skin and mucous membranes. and (3) splenomegaly.

SUMMARY.

1. In the venous blood of a patient with Vaquez' disease normal values were found for the oxygen unsaturation (reduced hemoglobin), although the total hemoglobin and oxygen capacity were abnormally high. The carbon dioxide content was normal.

2. The color of the skin and mucous membranes of this patient was more reddish than blue.

3. It is proposed to call the color of the skin in polycythemic patients erythrosis in order to distinguish the condition from cyanosis.

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